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Distribution of gastrin in the body. R. W. KEETON and F. C. KOCH.

The gastrin extracts were obtained by adding 5 parts 0.4 per cent hydrochloric acid to one part tissue, heating to steam bath temperature, allowing to cool to and then to digest at room temperature for 24 hours. After filtering and concentrating the filtrate under diminished pressure, the proteins were removed by the addition of alcohol, the filtrate again concentrated under diminished pressure and extracted with hot absolute alcohol to remove vaso-dilatins. The insoluble residue was dissolved in water and diluted so that 1 cc. of the final solution represented 4 to 5 grams fresh tissue.

The preparations were injected intramuscularly into gastric fistula and Pawlow stomach dogs. These methods showed gastrin to be uniformly distributed in the pyloric and fundus portions of the stomach while in the cardiac area slightly less active products were obtained. Gastrin was found in lower concentrations in duodenal mucosa and in traces in the oesophagus. Smooth muscle, pancreas and submaxillary gland by the same methods gave inactive preparations. Brain tissue gave an extract causing slight increase in the volume of juice with the same acidity, but with a decrease in peptic activity. The results lead the authors to believe that they are dealing with a specific substance found in the gastro-intestinal tract, but localized chiefly in the stomach. This substance gives rise to a true secretion in contra-distinction to the brain extracts which probably cause vaso-dilation only.

The relation of the hunger contractions of the stomach to the normal digestion movements. F. T. Rogers and L. L. Hardt.

Light on this question was sought in two ways; first graphic registration by the rubber balloon method of the activities of the stomach continuously from a time just after a meal until the hungry contractions were felt; second, X-ray observation of the hunger contractions using a bismuth-coated balloon with simultaneous graphic registration of the contractions. The first work was carried on with man and dogs, the second with dogs.

With the first method we find that there is present in the cardiac end of the stomach, in the absence of inhibiting nervous influences, a slow weak tonus rhythm which as digestion proceeds and the stomach empties itself becomes stronger and more rapid, culminating in the hunger contractions of the empty or nearly empty stomach. This tonus rhythm may be seen during the first hour after a meal. It is readily inhibited by nervous disturbances.

With the X-ray it may be seen that the weak hunger contractions are contractions of the fundus accompanied by a strong peristaltic wave which beginning near the cardia passes over the fundus. More vigorous hunger contractions are strong, rhythmical contractions of the whole fundus. If these contractions are peristaltic, their rate of advance is too rapid to be seen by this method. With the balloon in the pyloric end of the stomach only peristaltic contractions were seen.

The development of a tunicate without a nervous system. Ida H. Hyde.

From embryos and larvae of different ages of Ammaroecia, one of the Tunicates found in Woods Hole, up to date, five sets of experiments were made, controls kept, and material fixed and stained for further work.

The following is a preliminary résumé of the experiment and the results.

1. From free swimming larvae, or

2. From young embryos the tail or its anlage were removed. The organisms developed quite normally but were smaller than the control.

3. From free swimming larvae, or

4. From young embryos the tail and the nervous system or their anlage with the sense organs were removed. The results were small abnormal organisms, in which the heart began to beat and the siphons contracted slowly and incompletely. The investigation had not proceeded far enough to enable me to state that the heart reversed its rhythm spontaneously.

5. From embryos the nervous system in part or whole and the heart anlage were removed.

The resulting cell complex developed into an abnormal structure. The heart siphons and part of the digestive tract were missing. The cells that were left uninjured were capable of continuing their growth in a suitable environment up to a certain degree.

The influence of light on reproduction in Vorticella. IDA H. HYDE and CHRISTINE SPREIER.

Encysted Vorticellae were transferred by means of a Barber capillary pipette to a hanging drop infusion. When the zooids emerged from the cysts they were placed in a drop of the culture media on a hemocytometer micrometer slide. This was kept in a moist chamber. The Vorticellae of each series of the same age, were kept under the same conditions of temperature, moisture, food, and intensity of light but under different colored rays, of different degrees of intensity for at least three days. Colored glass, gelatin films and rays reflected from pure monochromatic colored cards both in bright and dim light were employed in studying the influence of colored light. For studying the influence of the intensity of light the Vorticellae were placed at different distances from the source of sun or electric light or back of smoked glass covers. The heat rays were cut out by water placed in parallel sided glass vessels in the path of the beams of light. From the results obtained we concluded that:

1. Vorticellae exposed to daylight increased more rapidly in number on bright sunny days than on dark cloudy ones.

2. On an average 25 developed one-half meter, 10 one meter from the source of light and 2 in the dark, from one Vorticellae in 24 hours.

3. The average increase in bright light was 40 from green, 29 under yellow, 26 under white, 17 under red and 13 under blue.

In dim light it was 5 for yellow, 3 for green, 2 each for white, blue and red, from one Vorticellae in 24 hours.

4. We conclude that the stimulating effect on the reproductive power of Vorticella increases up to an optimum intensity of light rays, with the intensity of the light and that the bright luminous rays of yellow and green are more effective than the red and blue ones.

The relation between the erythrocytes and the haemoglobin to the oxygen tension of the respired air. H. C. DALLWIG, A. C. KOLLS, and A. S. LOEVENHART.

The objects of the work:

1. To further elucidate the proposition that decreased oxidation leads initially to stimulation.

2. To determine definitely the cause of the increases in erythrocytes and haemoglobin at high altitude.

3. To determine the relative susceptibility of the respiratory center and the red bone marrow to decreased oxidation.

4. To form a part of a general study of the relation of oxidation to functional activity.

Methods:

First. We have worked at atmospheric pressure keeping the animals in an atmosphere of oxygen and nitrogen of definite and constant composition but varying the concentration of oxygen and nitrogen in different experiments.

Second. We have kept animals in rarified atmospheres, the degree of evacuation of the chambers yielding the same oxygen tension as in the experiments at atmospheric pressure.

The animals used were dogs, guinea-pigs and principally rabbits.

Results:

1. Lessening of the oxygen tension in the respired air leads to an increase in the erythrocytes and haemoglobin.

2. This increase is due to increased activity of the red bone marrow as is shown by a microscopical study of the bone marrow, by the occurrence of a large number of basophilic macrocytes in the blood and by the fact that the haemoglobin and erythrocytes do not increase proportionately.

3. The changes occur only if the oxygen tension of the respired air is reduced below 14 per cent of an atmosphere. It was found by Haldane and Priestley that when the oxygen tension of the respired air falls to about 13 per cent, the respiratory center is stimulated by oxygen want. Hence the respiratory center and the red bone marrow are apparently equally sensitive to oxygen want.

4. It is immaterial whether the lowering of the oxygen tension is brought about at atmospheric pressure by keeping the animals in an atmosphere poor in oxygen and rich in nitrogen or whether the oxygen tension of the respired air is lowered by a partial evacuation of the respiratory chamber.

5. The carbon dioxide tension in the respired air is a factor of but small or no importance.

6. The increase in erythrocytes and haemoglobin occurs in animals which are naturally anaemic as well as in animals with a high blood count.

7. The increase in the haemoglobin and erythrocytes occurs only after three to seven days and the return to the normal blood count is slow and gradual, often requiring two months.

The comparative rate of the oxidation of enzymes and their corresponding pro-enzymes. W. E. BURGE.

The object of this investigation was to determine if there was any difference in the rate at which pepsin and pepsinogen, trypsin and trypsinogen, respectively, were destroyed by oxidation.

The solutions of pepsin and pepsinogen were practically identical in strength after the activation of the pepsinogen. 150 coulombs of electricity were passed through 5 cc. of the solution of pepsin. Polarization was prevented by shaking the electrolytic cylinder. The amount of oxygen liberated by the passage of this amount of current was sufficient to oxidize practically all of the pepsin. The same amount of current was passed through an equal quantity of pepsinogen solution. The peptic activity of this solution was decreased by about 20 per cent. The conclusion is drawn that pepsinogen is more resistant to oxidation than pepsin.

The trypsinogen in 5 cc. of pancreatic juice was converted into trypsin by the addition of 2 drops of enterokinase. The amount of oxygen liberated by the passage of 150 coulombs of electricity through this solution practically destroyed its activity. A similar amount of electricity was passed through 5 cc. of pancreatic juice in which the trypsinogen had not been activated. Upon subsequently activating this electrolyzed solution it was found that the oxygen liberated by the passage of this amount of current had decreased its tryptic activity about 75 per cent. The conclusion is drawn that trypsin is more easily oxidized than trypsinogen.

On the concentration of sodium chloride in the plasma and its relation to the rate of excretion in normal and diabetic man. FRANKLIN C. MCLEAN.

The numerical laws governing the rate of excretion of sodium chloride formulated by Ambard and Weill (*Jour. de Phys. et Path. Gen.*, 1912,

vol. xiv) have been confirmed by us in numerous observations on normal individuals. The normal threshold for sodium chloride is 5.62 grams per liter of plasma, and when the concentration falls below this point excretion no longer occurs. Above this point the rate of excretion varies directly as the square of the excess of sodium chloride above 5.62 grams per liter. The excess may be calculated from the rate of excretion by the following formula, which corrects for the variable factors of the weight of the individual and the concentration of sodium chloride in the urine, as Ambard and Weill have shown that when the concentration in the plasma remains the same, the rate of excretion varies inversely as the square root of the concentration in the urine.

$$\text{Excess over } 5.62\ (\epsilon) = \sqrt{\frac{D \times \sqrt{\frac{C}{14}} \times \frac{70}{Wt}}{79.33}}$$

D = daily output, in grams
C = concentration of NaCl in urine, grams per liter
Wt = weight of individual, in kilos

The limit of error in applying this formula in normal individuals is about 0.08 gram per liter plasma, as determined by direct experiment in many individuals.

In eighteen diabetic patients examined about half were found to have a normal excretion of sodium chloride, as determined by comparison of the concentration of the plasma with the rate of excretion by the above formula. With one exception, a patient who had also a severe nephritis with chloride retention, the remainder of the cases were excreting sodium chloride on a markedly diminished threshold. In some of the cases the amount found in the plasma differed from the amount calculated by as much as 0.75 gram per liter. These patients complained of salt hunger, apparently due to the low chloride content of the plasma. In one case edema occurred while the patient was on a carbohydrate-free diet, and was apparently unaccompanied by any kidney change. Chloride retention in the plasma occurred during this edema, and the condition returned to its former state on disappearance of the edema.

When the intake and output of sodium chloride were measured in individuals excreting chloride on a lowered threshold the output was found to be normal, though much diminished during the occurrence of edema.

The diastases of the blood. HUGH MCGUIGAN, and C. L. v. HESS.

If, as has been suggested as probable by ourselves and reported as a fact by others, diastases will dialyse through collodion, it favors the opinion that the diastases are free in the blood and not in the form of a pro-enzyme. Careful experiments, however, show that diastases will not dialyse. Even when concentrated and with little colloidal admixture—as in saliva—no dialysis will take place through collodion in six or eight hours.

The injection of starch into the circulation causes an increase in the amount of blood sugar. This has been taken as evidence by some that diastase is present free in the blood. Against this opinion, amongst others, the following objections have been raised.

1. Many solutions cause hyperglycemia when so injected.

2. In most cases the amount of starch injected can not account for the persistent increase in the sugar.

3. The apparent enzymotic action may be the result of injury and consequent unnatural liberation of enzyme.

4. That the action is due to living cells and not enzymes.

In reply to these objections we may say that so far as we have found:

1. Colloidal solutions—unless they are sugar formers—reduce rather than increase the blood sugar, when injected intravenously.

2. That the amount of starch injected may account for the rise in sugar.

3. That the enzymotic action is not due to injury—because injection of water and other injurious agents does not increase the enzyme concentration of the blood, as measured by v. Hess' method, and old blood is less active than fresh, and becomes still less active with time.

4. This we can not answer satisfactorily but think it is not cell action because the starch paste would first have to enter the cell, which is impermeable, and if once in and hydrolyzed, we know of no normal instance where the sugar is again excreted as such, but instead is used by the cell. The enzyme concentration of the liver is less than the blood. which indicates that the utilization of sugar is cellular while the hydrolysis of starch to sugar is enzymotic.

Essentials of v. Hess' method to compare the relative strength of diastase in solutions.

1. Solutions required. (1) A 1 per cent soluble starch solution which must be clear.

(2) A 0.002 per cent solution of iodine made when needed by dilution of a freshly prepared 1 per cent iodine in 3 per cent KI.

The determination should be made in triplicate at 37°C. as follows: Keep all solutions in a water bath and have temperature of all the same before mixing. Place 10 cc. of the starch paste in each of three equal sized test tubes. In each test tube leave a separate pipette which will give the same size of drop in each case. Place 1 cc. of the solution whose diastatic strength is to be determined in each of the test tubes. Keep the time exactly and test for the disappearance of the color by placing exactly 1 drop of the digesting mixture on a white test plate and add to it exactly 1 cc. of the iodine solution. Repeat every minute when near the end point. By this method, when carried out as directed, the relation of the iodine to the starch is not varied and very accurate results can be obtained. The diastatic content of the blood of the dog varies but very little from day to day. The end point is usually reached in about thirty-five minutes.

The action of pituitrin on the mammary gland. W. L. Gaines.

The action of pituitrin on the mammary gland was followed by observation of, first, the effect on milk secretion, and, second, the change in internal volume of the gland. In the volume method a cannula was inserted in the teat of a goat, the gland inflated with air and connected with a manometer recording on a kymograph.

Upon intravenous injection of pituitrin the active gland, milked dry by hand, shows an abrupt decrease in volume and increase in pressure, followed by a gradual return to the initial volume and pressure. A repeat dose gives a similar result. This repetition excludes milk secretion as a possible cause of the volume and pressure changes, since a repeat dose causes no secretion of milk. The non-lactating gland shows no response on injection of pituitrin, and this is true, also, when the other gland of the pair is still functional. The absence of any response in the inactive gland excludes vaso-motor changes as a possible cause of the results observed in the case of the active gland. Pituitrin causes a contraction of the gland musculature when the gland is functional, but has no effect when the gland is inactive, and the difference in sensitiveness of the gland musculature in the active and inactive gland is not due to the presence or absence of any particular substance in the circulating blood stream.

In normal nursing of the dog the musculature of the gland is involved and is excited reflexly by the nursing of the pups, so that the rate of secretion is at first slow, increasing to very fast, then decreasing to zero. At the zero stage pituitrin causes no further secretion. The reflex

mentioned is partially or completely inhibited by ether anesthesia, so that the nursing pups are able to secure only a small portion or none of the normal yield, and in this condition injection of pituitrin causes an immediate secretion of milk, to equal the normal yield. A repeat dose causes no further secretion.

In the goat hand milking at equal time intervals gives normally a fluctuating yield. Injection of pituitrin immediately following a low yield causes a marked further secretion; but, following a high yield it causes little or no further secretion. The effect of pituitrin on milk secretion upon injection immediately following natural nursing or artificial milking is dependent on the efficiency with which the muscular mechanism of the gland has been brought into play by the nursing or milking to remove the accumulated milk from the gland.

The influence of depancreatization upon the state of glycaemia following the intravenous injection of dextrose in dogs. I. S. KLEINER and S. J. MELTZER.

In former experiments (see *Proceedings of the American Physiological Society*, vol. 33, 1913, p. xxvii) it has been shown that after the intravenous injection of large amounts of dextrose (4 g. per kilo) into dogs the sugar rapidly disappears from the blood stream so that after $1\frac{1}{2}$ hours after the end of the injection the blood-sugar falls nearly to its original figure. In the present experiments the same procedure was carried out on completely depancreatized dogs. In these cases the blood-sugar did not fall to its original value or near it; at the end of $1\frac{1}{2}$ hours it was on the average more than twice as high. The following is a comparison of the average figures:

	BLOOD-SUGAR			
	Before injection	End of injection	$1\frac{1}{2}$ hours after end of injection	Dextrose in urine, % of amount injected
Normal (5)	0.20	0.79	0.27	> 43
Depancreatized (9)	0.38	1.19	0.86	49 (uncorrected for "diabetic" sugar).

A similar difference was observed also in nephrectomized dogs.

It is claimed by some investigators that the glycaemia following depancreatization is due to an over-production of sugar. It is evident that the hyperglycaemia in our cases of depancreatization can not be due to such a factor. We shall not discuss here whether our results

can be adequately explained by the assumption that the removal of the pancreas causes a decrease in the consumption of dextrose by the body tissues. We wish, however, to indicate that some of our facts hint at the possibility of a change in the permeability of the endothelia of the circulatory apparatus as a factor in the results of depancreatization.

Recuperation. Nitrogen metabolism of a man when ingesting successively a non-protein and normal diet after a seven-day fast. F. D. ZEMAN, JEROME KOHN and PAUL E. HOWE.

This is the third[1] of a series of experiments concerned with changes in metabolism of man following the ingestion of food after a fast. In the recuperation periods (4 day) of this experiment non-protein and normal diets were fed; the preliminary and final diets were the same. The non-protein diet consisted of cane sugar, clarified butter, an alkaline salt mixture and agar agar having an approximate daily fuel value of 3500 cal. Determinations were made of the body weight, and the excretion of water, total N, urea, ammonia, creatine and creatinine in the urine.[2]

The excretion of the various urinary constituents followed the usual course during the fast; the total N excretion on the seventh day was approximately 10 grams and creatine appeared on each day. The ingestion of a calorifically sufficient non-protein diet resulted in a decrease of the nitrogen excretion which became constant on the third and fourth days. Minimum values obtained on the second day of feeding, were as follows: total N 3.56 grams, urea-N 1.59 gram, ammonia-N 0.54 gram, creatinine-N 0.61 gram, creatine-N 0.05 gram. A relatively high ammonia-N excretion (0.72 gram, 17.4 per cent. of the total N) occurred on the third day. Normal conditions tended to return in the final period while the subject was retaining nitrogen. A lowered absolute and relative ammonia-N excretion was observed.

The daily nitrogen excretion through the feces during the non-protein period was 0.50 gram.

A comparison of the changes in body weight and the nitrogen balances shows an increase in body weight during the non-protein feeding period accompanied by a loss of nitrogen while the reverse occurred in the final period. The initial increase in weight upon the ingestion of food is the

[1] The first two experiments were reported by Howe, Mattill and Hawk, Jour. Amer. Chem. Soc., 33, p. 568, 1911, and Howe and Hawk, Proc. Amer. Soc. Biol. Chem., 2, p. 65, Jour. Biol. Chem., 11, p. xxxi, 1912.

[2] Variations in factors associated with changes in the urinary acidity are reported in another connection.

result chiefly, of the retention of water and to a smaller degree of non-nitrogenous food substances.

Apnoea as an after-effect of pulmonary distention, and its dependence upon the vagus nerves. T. S. GITHENS and S. J. MELTZER.

In recent years the conception became dominant, due especially to the investigations of Haldane and his pupils, that apnoea as an after-effect of distension of the lung is essentially of chemical origin, due to a reduction of carbon dioxid in the blood circulating through the respiratory center; this has been designated by them as "true apnoea." Furthermore, it was recently stated that there is no experimental evidence for a possible claim that true apnoea *could* depend exclusively upon the intactness of the vagus nerves.

Of our recent investigations of this subject we wish to mention here the following three facts observed by us (and recently demonstrated at a meeting of the Society for Experimental Biology and Medicine). 1. A fairly prolonged characteristic apnoea follows a short distension of the lungs in dogs *without any previous artificial respiration.* The duration of the apnoea depends, within certain limits, upon the degree of pressure used for the distension (Meltzer's pleural cannula was used for the graphic presentation of respiration). 2. The same apnoea after-effect can be obtained when air used for distention of the lungs contains 5 per cent CO_2. 3. No such apnoea after-affect can be obtained after both vagus nerves are cut.

These experiments demonstrate that the mere distension of the nerve endings of the pulmonary vagus without the aid of a chemical factor (acapnia) is capable of producing a prolonged apnoea as an after-effect of the mechanical stimulus. The restriction of the term "true apnoea" to a condition produced exclusively by chemical changes does not seem to be well founded.

Experimental hyperthyroidism. W. B. CANNON, C. A. L. BINGER and R. FITZ.

Interest in the bodily changes during or following emotional excitement led us to enquire concerning the nature of certain diseases often reported as having emotional origin. We proceeded on the theory that repeated emotional experiences might lower a naturally high neurone threshold and thus result in frequent stimulation of parts which normally are only occasionally roused to special activity. To test the effect of over stimulation two of us (W. B. C. and C. A. L. B.) fused in the cat the anterior root of the right phrenic nerve with the right cervical

sympathetic cord. Thus after regeneration had occurred, there was delivered to neurones in the superior cervical ganglion a volley of impulses every time the animal breathed. The operations were performed early in May. In October four of six animals were still alive. All had peculiar symptoms. There was marked tachycardia—the average heart rate in 36 observations on normal cats was 165, in 30 observations on these animals it was 222. Though fed like normal animals they had loose movements of the bowels. They suffered from falling of the hair from the neck and back, and they acted as if afflicted with pruritus of the head and toes. They were unusually excitable, as indicated by rushing away when taken in hand or petted. One of us (R. F.) has studied the basal metabolism, and found that the average heat loss per kilo per 24 hours in normal adult cats is 44 calories; in three of the four experimental animals it was 66 calories,[1] and in one (in all ways the most profoundly altered animal) it was 112 calories—an increase over the normal of more than 150 per cent. This animal, after very rapid loss of weight, has died. At autopsy the adrenal glands were found nearly three times the average weight. In dim light the pupil in these animals was larger on the operated side, and in one of them exopthalmos and respiratory hippus have developed on that side. These symptoms are, in the main, characteristic of exophthalmic goiter, as seen in man.

The observations on these four animals is preliminary to a more extensive study of the subject. The method of using the phrenic as a source of stimuli is now being applied not only to the thyroid gland but also to other organs innervated by the autonomic system. We have planned a series of studies on overaction of the autonomic, to be carried out by use of this method.

Oxidation in the erythrocytes of the goose. J. F. McClendon.

The oxidative coloring of leucobases by erythrocytes was increased on laking, due to mixing of oxyhaemoglobin with the leucobase. (See my paper on oxyhaemoglobin below).

Colorimetric methods in general use for measuring intracellular oxidations were discarded, since oxyhaemoglobin was found to oxidise many of these substances. The erythrocytes (in Ringer) were shaken with air in a flask connected with a water manometer, and immersed in a thermostat. The oxygen absorbed was equal to the oxygen used by the cells (since the haemoglobin was saturated with O_2 at the beginning).

[1] The metabolism of one of the three has since gradually risen—77, 87, 98, 109, 103 calories—with corresponding loss of weight.

Numerous experiments, made under a great variety of conditions to determine whether induction shocks increase intracellular oxidations, failed to show such increase. If induction shocks affect the cells physiologically, it is probable that strong shocks affect them more than weak ones. It was found that the electric conductivity was slightly increased by strong shocks, without laking any of the cells. This suggests that one might obtain an increased coloring of a leucobase by erythrocytes subjected to the shocks, since the increased permeability might allow the leucobase to reach the oxyhaemoglobin more rapidly. Such a result was obtained by R. Lillie.

In the oxidation experiments, the manometer readings are greatly affected by changes in temperature and barometric pressure. In order to equalize these, a brass tube 2 *m.* long and 7 mm. bore was coiled in the thermostat and connected with a Marey's tambour, the platinum pointer of which, dipping in mercury, made and broke a heat-controlling electric circuit. Readings were made in this "barostat," whereas the oxidation took place in a thermostat independent of temperature. The "barostat" was sensitive to a thousandth of a degree, but might change 6° due to change in barometric pressure.

The increase in permeability of the frog's egg at the beginning of development as determined with the nephelometer. J. F. McClendon.

Three years ago, I observed that the unfertilized frog's egg could be made parthenogenetic by a momentary electric shock, and gave reasons for supposing that the electric shock (or the spermatozoon in normal fertilization) increased the permeability of the egg. Recently, I proved this supposition to be correct. The permeability of the unfertilized egg to NaCl was found to have increased on stimulating the egg with an electric shock (which caused it to begin normal development).

Several methods were tried for the quantitative estimation of sodium ions, but the results with such small quantities would not be considered trustworthy had they not tallied with the more certain results on the determination of chlorine ions with the nephelometer.

Lot 1 was stimulated by an electric shock from clean platinum electrodes (in about one minute all of the eggs had turned the black pole upward; 23 hours later first cleavage began) and lot 2 used as a control. Twenty cubic centimeters of H_2O were added to each lot and at the end of one hour this water was analyzed. There was more $Na+$ and $Cl-$ in the water from the stimulated eggs than in the control. Whether this increase in permeability is the cause of development has

not been determined. It is not restricted to the frog's egg, however, since I found the same true of the sea urchin's egg, a fact which has been confirmed by Gray, at Plymouth.

The unfertilized frog's egg placed in tap water or distilled water continues to swell until death ensues. This death is probably caused by the swelling and the latter by the osmotic pressure of the soluble substances contained within it. The increased permeability allows the escape of NaCl and lowers the internal osmotic pressure, thus retarding the swelling and preserving the life of the egg.

Some experiments on the oxidising power of oxyhaemoglobin. J. F. McClendon.

Many leucobases + H_2O_2 are colored on the addition of blood pigments, but some of these bases are oxidised on the addition of dog's erythrocytes, in the absence of H_2O_2, especially if the solution is slightly more alkaline than the blood. Alpha-napthol, aloin, or paraphenylendiamide is colored rapidly—benzidine or guaiac, not so. The same oxidising power is possessed by oxyhaemoglobin, recrystallised five times, so it is not due to so called "oxidases" as an impurity. Methaemoglobin recrystallised seven times, oxidised these substances.

Some measurements were made with the Dubosque colorimeter, on the rate of oxidation of Vernon's "substrate" ($\frac{1}{100}$ normal alpha napthol and paraphenylendiamide, slightly alkaline). Solutions of dog's erythrocytes, oxyhaemoglobin crystalized five times and methaemoglobin crystallized seven times were made up to be equivalent to 5 per cent of blood. These solutions were mixed with equal volumes of substrate and studied in pairs, with an arrangement to compensate for the difference in color of the blood pigments. The solution of erythrocytes did not oxidise the "substrate" faster than the oxyhaemoglobin or the methaemoglobin.

We thus see the necessity of removing the last traces of blood pigments in studying the so-called "oxidases" colorimetrically.

It is a recorded fact that blood charcoal oxidises oxalic acid to CO_2 and H_2O, whereas other charcoal is less effective, or not at all. This might be due to the presence of iron and adsorption surfaces. Since Warburg has shown that adsorption plays a rôle in the oxidative power of oxyhaemoglobin, we might expect that the blood pigment would behave differently inside and outside of the corpuscle, since it is too concentrated within the corpuscle to exist in simple aqueous solution and probably has a different aggregation state which would affect adsorption.

Lactic acid and sugars are not oxidised to CO_2 and H_2O, by blood charcoal or by oxyhaemoglobin.

The harmful effect of a vegetable diet. CARL VOEGTLIN.

Feeding experiments with natural vegetable foods in monkeys, white mice and rats, hogs, and fowls are reported with the following results: (1) An exclusive diet of cereals of good quality such as wheat, corn, barley, oats, millet, etc., is injurious to some mammalia and leads sooner or later to the death of these animals. (2) An exclusive diet of some fresh vegetables, such as carrots, Irish potatoes and sweet potatoes has the same effect. (3) Legumes such as beans and peas seem to be insufficient for the maintenance of life if forming the only diet of mice and rats. (4) Fresh beef, ox liver, eggs and milk, if added in sufficient amounts to the vegetable food will protect the health of these animals. (5) A mixed vegetable diet composed of cereals, legumes and fresh vegetables is inadequate for the maintenance of life in mice and certain other mammalia. (6) Fowls can live in perfect health for a long period on an exclusive diet of corn, wheat and other cereals. They die if put on an exclusive diet of corn oil cake meal (absence of certain vitamines). In those animals, which finally died as a result of an exclusive vegetable diet, symptoms were noticed pointing to a pathological condition of the central nervous system and the alimentary canal (paralysis, strychnine-like convulsions, diarrhoea or constipation). Marked histological changes were found in the organs of these animals. The addition of extracts of beef liver and yeast (vitamines) to the vegetable diet does not seem to have any effect on the occurrence of pathological symptoms and subsequent death of the animals. When certain inorganic salts (calcium and sodium phosphate) are added to the corn diet, the life of the animals (mice) is very much prolonged. Our present knowledge of the nutritive value of most of the natural vegetable foods is very limited. The reported experiments demonstrate the inadequate composition of these foods and the harmful effect they may produce in animals if fed exclusively. That a mixed diet composed of both vegetable and animal foods is undoubtedly less apt to be harmful under normal conditions is well demonstrated by everyday experience.

The path of conduction between the sino-auricular and the auriculo-ventricular nodes. J. A. E. EYSTER and W. J. MEEK.

All recent work seems to show that in the normal cardiac cycle electrical negativity is first to be observed in the sino-auricular node.

A few hundredths of a second later the same condition becomes manifest in the auriculo-ventricular node. The work here reported has been an attempt to find the path by which this wave of negativity passes from one node to the other. Our methods have been electrical and have consisted in determining by string galvanometers the actual and relative times at which various regions of the supraventricular parts entered into activity.

In the first series of experiments differential electrodes of a modified Clement type were placed on the S-A node, the body of the right auricle and on the A-V node. It was found that in many cases the A-V node became active before the body of the auricle and that in the cases in which the reverse was true the time interval was apt to be very short. In reversed rhythms the S-A node was often negative before the auricle. These results have been interpreted to mean that conduction between the two nodes is not usually at least by way of the right auricle.

In a second series of experiments a double circle of points around the S-A node were carefully compared with each other to find which first showed negativity. The rule is almost invariable that the venous side shows activity before the auricular. The region most often showing negativity soonest after the node itself is an area immediately adjoining the head of the node on the venous side.

In a third series the differential electrodes were placed as at first and then various cuts and ties were made around the S-A node. Interrupting tissue connections between the S-A node and the auricle always delayed sino-auricular conduction time but it had no other effect on the heart. Tieing the bundles of tissue which run off from the end of the sulcus terminalis was without effect. Each interruption of tissue on the venous side without exception delayed the conduction time between the S-A and A-V nodes. More than one cut was apt to produce auriculo-ventricular rhythm.

Our results seem to indicate that the path of least resistance between the upper and lower auricular regions of specialized tissue lies on the venous side of the S-A node and does not involve the body of the right auricle. So far as can be told at present this path is probably a diffuse one.

The metabolism of the resting nerve and its correlation with the direction and rate of nerve impulse. SHIRO TASHIRO.

There is a gradient of carbon dioxide production in the unstimulated nerve. This gradient of chemical condition is parallel to the direction

of the normal nerve impulse, and not to the direction of development of the fiber from the nerve cell. Many experiments made on various kinds of "pure" nerve fibers, including sensory dendrites, enable us to generalize this by saying that the normal nerve impulse, in the resting nerve, passes toward a point of lower carbon dioxide production.

There seems to be a close relation between the rate of nerve impulse and the production of carbon dioxide in the resting nerve, if one compares corresponding nerves from different animals. The data for such a generalization must necessarily be cumulative. The limited data we have secured indicate that the nerve which gives off more carbon dioxide in the resting state conducts the nerve impulse more quickly.[1]

There are several conditions which affect the rate of nerve conduction, e.g., temperature and change in concentration of electrolytes in a solution surrounding the nerve. Mayer found that the rate of nerve conduction in the subumbrellar regions of Medusa Cassiopeia increases about 5 per cent in sea water diluted with distilled water (9:1), while it decreases 50 per cent in 50 per cent sea water. By substituting 0.9 molecular dextrose for distilled water he demonstrated that the change in the rate of nerve impulse in diluted sea water is not due to the decrease in osmotic pressure, but to the change in concentration of the electrolytes. If, under these conditions which decrease the rate of the nerve impulse, a measurement of carbon dioxide production is made on a thin layer of regenerating ectoderm tissue before the muscle regenerates, we find that there is a parallelism between the rate of nerve conduction and carbon dioxide production.

The temperature coefficient of velocity of the nerve impulse is known to be greater than that of most purely physical processes. We find that the temperature effect on carbon dioxide production from non-stimulated nerves (the claw nerve of the Limulus) is of about the same magnitude as that of the velocity of the nerve impulse.[2]

These facts seem to indicate that there is a definite relation between the metabolism (as measured by carbon dioxide production) in the resting nerve and functional activity in the nerve fiber, including the direction and rate of the nerve impulse.

[1] The reason why these relations will not hold if we compare non-medullated with medullated fibers will be published later.

[2] I must add here that the temperature coefficient of the velocity of nerve impulse in the claw nerve of Limulus has not yet been worked out.

FEEDING EXPERIMENTS ON RATS

III. A Further Contribution to the Knowledge of Organs With an Internal Secretion

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A series of experiments is being carried on in this laboratory to study the influence upon growth and development of the various internally secreting glands. These glands are being fed to white rats in stated portions, at regular intervals.

The experiments have not proceeded far enough to make a detailed report on the effect of all the organs used. Here merely a preliminary account of some observations on the thyroid treated animals will be given.

Fresh beef thyroid was used and given in portions small enough to keep the animals in fairly good health. In the earlier experiments too large a dose of thyroid had been given, 5 g a week, so that the animals very soon showed all the well-known signs of hyperthyroidism usually leading to death. The dose was gradually cut down to 1 g a week, in other experiments to 1 g in 5 days. Even with such small doses the symptoms of hyperthyroidism were sometimes slightly noticeable, however, the animals kept so well that they were able to produce offspring. After the dose of thyroid had been cut down considerably, the animals not only bred, but the young were also strong enough to keep alive.

In the following list four matings will be described in detail, the offspring of which are still living.

I. ♂t × ♀t[1]

HISTORY OF FATHER

Born July 21, 1913.
Thyroid feeding began January 6, 1914.

1. Mating: ♂t × ♀t 1/26–2/16[2] 1914; died; no pregnancy.
2. Mating: ♂t × ♀t 2/19–3/10, 1914; ♀ died; no pregnancy.
3. Mating: ♂t × ♀t 3/21–5/15, 1914; no pregnancy.
   Thyroid feeding stopped 5/15, 1914.
4. Mating: ♂t × ♀n 5/15–6/8, 1914. 10 young 6/10. Frail, died within a week.

HISTORY OF MOTHER

Born December 31, 1914.
Thyroid feeding began 3/24, 1914.
Thyroid feeding stopped 6/10, 1914.

Final mating which gave living offspring

♂t × ♀t 6/12–7/6, 1914. 3 young born 7/9, 1914. Young are living now. They are much smaller (figs. 1, 2) than the normal rats of the same age (fig. 3). At the age of 152 days they weighed 97 g, 101 g, 190 g resp. Normal rats of this age weigh from 150–220 g.

SUMMARY OF CASE I

(a) While under treatment the father was bred unsuccessfully to 3 treated ♀.

(b) After discontinuation of the thyroid treatment the father was bred to a non-treated ♀. 10 young were born after 26 days, all so frail that they died within a week.

(c) One month after the thyroid treatment had stopped, the father was bred to the mother, the thyroid treatment of which ceased on the mating day. The results were 3 undersized young born 54 days after the father and 29 days after the mother had received the last dose of thyroid.

In this case father and mother received thyroid *before* mating.

II. ♂t × ♀n.

HISTORY OF FATHER

Born January 28, 1914.
Thyroid feeding began 3/24, 1914.
Thyroid feeding stopped 6/10, 1914.

HISTORY OF MOTHER

Born February 26, 1914.
Normal feeding.

[1] t = treated. n = normal.

[2] 1/26–2/16 means: ♂ and ♀ were kept together from January 26 until February 16.

Final mating which gave living offspring

♂t × ♀n 6/12–7/6, 1914. 4 young born July 10, 1914. 2 young died after 3 days; 2 young living, undersized (figs. 4, 5).

SUMMARY OF CASE II

The father immediately after the thyroid treatment had ceased, was bred to the non-treated mother. 4 young were born 30 days after the last thyroid dose had been given. 2 young died soon, 2 undersized ones are living.

In this case the father only received thyroid *before* mating.

III. ♀t × ♂n.

HISTORY OF FATHER

Born December, 1913.
Normal feeding.

HISTORY OF MOTHER

Bought November, 1913, about 3 months old.
Thyroid feeding started 1/6, 1914.

1. Mating ♀t × ♂t 3/21–5/15, 1914; no pregnancy.
Thyroid feeding stopped 5/15, 1914.

Final mating which gave living offspring

♀t × ♂n 5/15–9/6, 1914. 6 young born 9/6, 1914. 2 young die 10/13, 1914. 4 living, small size (figs. 6, 7, 8) control figure 9. At the age of 94 days they weighed 46, 50, 58, 83 g resp. Normal rats of this age weigh from 120–150 g.

SUMMARY OF CASE III

(a) While under treatment the mother was bred unsuccessfully to a treated ♂.
(b) The treated mother immediately after the thyroid treatment ceased was bred to the non-treated father. 6 young were born 113 days after the last thyroid dose had been given. 4 young, smaller than the normal, are living.

In this case the mother only received thyroid *before* mating. She required three months to recover from the thyroid influence.

IV. ♂t × ♀n.

HISTORY OF FATHER

Born July 24, 1913.
Thyroid feeding started 3/21, 1914.

1. Mating ♂t × ♀t 3/21–5/15, 1914; no pregnancy.
Thyroid feeding stopped 5/15, 1914.

HISTORY OF MOTHER

Born May 26, 1914.
Normal feeding.

1. and 2. Matings: Bred to two normal males successively. 6 young and 8 young.

Final mating which gave living offspring

♂t × ♀n 5/15–9/10, 1914. 3 young born September 10, 1914. Undersized. At the age of 90 days they weighed: 52, 63, 99 g resp. Normal rats at this age weigh from 120–150 g.

SUMMARY OF CASE IV

(a) While under treatment the father was bred unsuccessfully to a treated ♀.
(b) The normal mother was bred successfully to 2 normal ♂.
(c) The treated father immediately after the thyroid treatment had ceased was bred to the normal mother.
3 young were born 117 days after the last thyroid dose was given.

In this case the father only received thyroid *before* mating. He required three months to recover from the thyroid influence.

To this list must be added a number of cases in which young were born alive, but died after puberty.

V. ♂t × ♀n.

HISTORY OF FATHER

Bought January, 1913. About 3 months old.
Thyroid feeding started January 13, 1913.

1. Mating: ♂t × ♀t 4/7–4/28, 1913; no pregnancy.

HISTORY OF MOTHER

Bought January, 1913. About 3 months old.
Normal feeding.

Final mating which gave offspring

♂t × ♀n 4/7–4/28, 1913. 5 young born 5/1, 1913.

HISTORY OF THE YOUNG

Born May 1, 1913.

1. Young died after 9 days. Very frail.
4 other young started on thyroid diet October 30, 1913. They were all undersized and rather feeble. One dragged the hind legs. When 84 days old they weighed: 57 g, 58 g, 60 g, 66 g, resp. Normal rats at this age weigh from 110–140 g.
2. Young died November 10, 1913.
3. Young died December 3, 1913.
4. Young died December 5, 1913.
5. Young died December 21, 1913.

SUMMARY OF CASE V

(a) While under treatment the father was bred successfully to a treated ♀.
(b) While under treatment the father was bred to the non-treated mother.
5 young were born 24 days after mating. 1 died 9 days old, 4 frail and undersized lived several months.

In this case the father only received thyroid up to the time of mating.

VI. ♂t × ♀t.

HISTORY OF FATHER

Bought January, 1913. About 3 months old.
Thyroid feeding started January 13, 1913.
1. Mating: ♂t × ♀t_3 4/7–4/28, 1913; no pregnancy.
2. Mating: ♂t × ♀n 4/7–4/28, 1913. 5 young 5/1, 1913 (case V).

Final mating which gave offspring

♂t × ♀t_3 5/26–9/14, 1913.
(Female in this successful mating is the same as in the first unsuccessful mating).
Thyroid feeding stopped June 26, 1913.

HISTORY OF MOTHER

Bought January, 1913. About 3 months old.
Thyroid feeding started January 13, 1913.
1. Mating: ♀t × ♂t_2 4/7–4/28, 1913; no pregnancy.

Final mating which gave offspring

♂t × ♂t_2 5/26–9/14, 1913.
Thyroid feeding stopped 6/26, 1913.

HISTORY OF THE YOUNG

7 young born September 14, 1913. Very frail and undersized. These young were started on thyroid October 31, 1913.

2 die November 10, 1913.
3 die November 12, 1913.
2 die November 15, 1913.

SUMMARY OF CASE VI

(a) While under treatment the father was bred unsuccessfully to the treated mother.
(b) While under treatment the father was bred to a non-treated ♀ (see case V).
(c) The treated father and treated mother (under a) were again mated. The thyroid treatment ceased 31 days after mating.
7 young were born 81 days after the last thyroid dose was given.

The treated parents required two months to recover from the thyroid influence.

VII. ♂t × ♀n.

HISTORY OF FATHER

Born July 21, 1913.
Thyroid feeding started January 6, 1914.

1. Mating: ♂t × ♀t 1/26–2/16, 1914; no pregnancy.
2. Mating: ♂t × ♀t 2/19–3/10, 1914; no pregnancy.
3. Mating: ♂t × ♀t 3/21–5/15, 1914; no pregnancy.

Thyroid feeding stopped May 15, 1914.

Final mating which gave offspring

♂t × ♀n 5/15–6/9, 1914. 10 young born 6/10, 1914. All die during June.

SUMMARY OF CASE VII

(a) While under treatment the father was bred unsuccessfully to 3 treated ♀.

(b) The treated father, the thyroid treatment of which ceased on the mating day, was mated to the non-treated mother.

10 young were born 26 days after the last thyroid dose was given. All died within 2 weeks.

In this case the father only received thyroid *before* mating.

VIII. ♀t × ♂n.

HISTORY OF FATHER

Born May 26, 1913.

1 and 2. Matings: ♂ bred to 2 normal females successfully. 8 and 7 young.

HISTORY OF MOTHER

Born July 21, 1913.

1. Mating: Bred to a normal male. 6 young born 1/26, 1914. Thyroid feeding started March 21, 1914.
2. Mating: ♀t × ♂t 3/21–5/15, 1914; no pregnancy.

Thyroid feeding stopped May 15, 1914.

Final mating which gave offspring

♀t × ♂n 5/15–10/14, 1914.

5 young born 10/14, 1914, all eaten up by 10/19, 1914.

SUMMARY OF CASE VIII

(a) The non-treated father was bred successfully to 2 non-treated ♀.

(b) Before thyroid treatment began the mother too was bred successfully to a non-treated ♂.

(c) While under treatment the mother was bred unsuccessfully to a treated ♂.

(d) Finally the treated mother, immediately after the thyroid treatment had ceased, was bred to the non-treated father.

5 young were born 151 days after the last thyroid dose was given.

In this case the mother only received thyroid *before* mating. She required over four months to recover from the thyroid influence.

These eight cases reported here as well as numerous others of which the detailed history cannot be given leave no doubt that the

excess of thyroid in the system greatly interferes with the breeding qualities of the animals. Twenty-four matings in which both the parents were treated resulted in failure, 2 in which the male alone had been treated and 4 in which the female alone received thyroid food, all in all 30 matings. Yet out of these 14 males, 7 had been tested and given offspring previously to the treatment, and out of the 16 females 9 had been tested and found fertile.

In a number of cases the female died before pregnancy began or before a live litter was born. The length of time these females were kept with the males is given in the following table:

1. ♀ dies after 2 days		Thyroid given after mating.
2. ♀ dies after 2 days		
3. ♀ dies after 7 days	no pregnancy	
4. ♀ dies after 19 days		
5. ♀ dies after 21 days		
6. ♀ dies after 21 days		
7. ♀ dies after 38 days		
8. ♀ dies after 57 days 6 fetuses		No thyroid given after mating.
9. ♀ dies after 67 days 7 fetuses		
10. ♀ dies after 107 days pregnant		
11. ♀ dies after 112 days pregnant		

The continuation of this table gives the time that elapsed between mating and birth of live litter:

12. Young born after 24 days ♀ *normal* All die early	No thyroid given after mating.
13. Young born after 26 days ♀ *normal* All die within two weeks	
14. Young born after 27 days ♂ fed on normal food full month previous to mating ♀ fed on thyroid 2 months only	
15. Young born after 28 days ♀ *normal* 2 die within a week	
16. Young born after 110 days 87 days after thyroid treatment	Thyroid feeding continued for 33 days after mating.

17. Young born after 113 days ♂ *normal* 18. Young born after 117 days ♀ *normal* 19. Young born after 151 days ♂ *normal*	No thyroid given after mating.

This enumeration of 19 matings shows that pregnancy did not set in until several weeks after the discontinuation of the thyroid feeding. Whether or not copulation without fertilization took place before that time, cannot be stated. Surely attempts to copulate were made by the several males.

In those cases in which the litter was born within less than 30 days after mating,[3] the mother was normal, viz., not fed on thyroid (Nos. 12, 13, 15). Still the young died soon after birth. In the one case in which both parents had been treated and yet pregnancy set in early (No. 14), the male had been fed on normal food one month previous to the mating, while the female had been treated with thyroid for two months only. In cases 17–19 one of the two parents was normal, but it took the treated partner over three months to recover from the thyroid influence.

Thus under no circumstances will the continuation of the thyroid treatment allow pregnancy to set in. Numerous matings of this kind resulted in failure. Also in case 16 fertilization did not occur until 63–65 days after the thyroid feeding was stopped.

The feeding to rats of fresh thyroid tissue shows its effect in three different ways:

1. When the dose is too large, all the well known symptoms of hyperthyroidization become evident, viz., emaciation, diarrhoea, muscular weakness and finally cachexia leading to death. The hair becomes yellowish, stands erect, sometimes falls out in patches, in short the entire coat looks ragged.

2. When the dose is so regulated as to keep the animals in apparently good health—the fur will always become shabby—then the animals do not breed. Not one mating of both parents treated gave any result, when the feeding continued after the animals had been placed together. Pregnancy was always

[3] The gestation period of the rat is from 21–23 days.

delayed, since fertilization did not occur until several weeks after the application of the thyroid had been discontinued.

3. Did pregnancy finally occur, it resulted

(a) In abortus,

(b) The young died soon after birth,

(c) In very late pregnancies, the young show a diminished tendency to grow. Although they are not especially frail, they keep in relative size behind the normally fed rats of the corresponding age.[4]

The question now presents itself, whether the symptoms enumerated under (2) and (3) are simply the consequences of the general weakening effect of the thyroid food (1), or whether the genital glands have been affected so as to injure the sex cells. From the various reports in the literature on the interaction of thyroid and genital glands, especially under pathological conditions, this question is justified.

It may at once be said that the delay of pregnancy (2) is in all probability due to the weakening effect of hyperthyroidization. It seems, however, that this cannot fully account for the effects of the thyroid treatment, as stated under (3). It is reasonable to hold the latter responsible for the death of the several mothers during pregnancy and the reduced vitality of the offspring of early pregnancies. But the slackened growth of the young of late pregnancies can hardly be solely due to the weak condition of the mother. It can surely not be due to that condition in those matings in which the father only had been treated, while the mother was a normal female. It seems rather, although at present this is a mere assumption, as if the tendency to grow, inherited from the two parents, had been checked in some way. Former experiments already reported and some under way clearly show that the rate of growth and differentiation of a generation can be influenced by the application of thyroid, but there

[4] There is considerable variation in size and weight between the several stocks of white rats raised in different laboratories. For information on this point I am much obliged to Professor H. H. Donaldson, of the Wistar Institute. I also refer to his various papers on this question. The thyroid treated rats do not in a given time reach the length and weight of our normal rats. Compare illustrations.

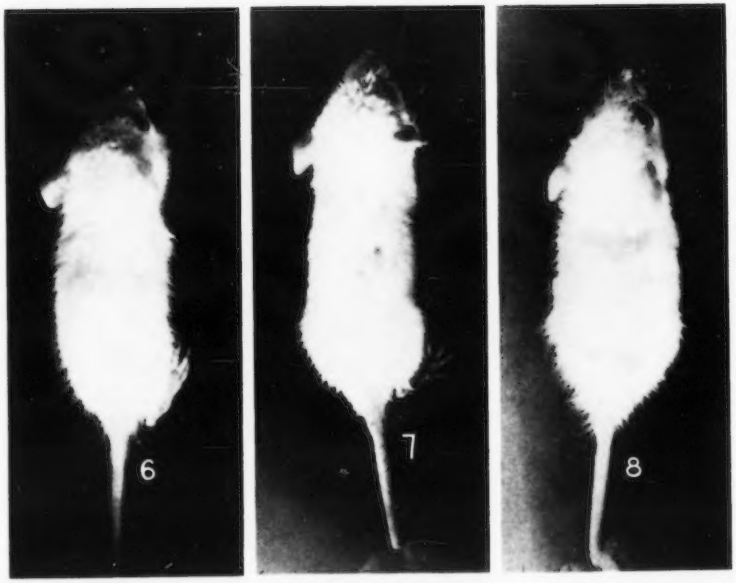
FIGS. 1 and 2. One-third reduction. 2 rats 97 days old. Father and mother treated with thyroid.

3

FIG. 3. One-third reduction. Normal rat 91 days old.

FIGS. 4 and 5. One-third reduction
treated with

2 rats 96 days old. Father
hyroid.



FIGS. 6-8. One-third reduction. 3 rats 38 days old. Mother treated with thyroid.

9

FIG. 9. One-third reduction. Normal rat, 34 days old.

is no evidence yet that this influence can be transmitted to the second generation. If it could be shown that the rats of the second generation, although stagnant in growth, differentiate and mature at the same rate as or faster than normal rats, the proof for the correctness of the above assumption could be given.

This preliminary report is supposed to state only some of the actual observations so far made. The histological data concerned will be reported, when the experiments will have been carried sufficiently far.

THE CHANGES IN THE CONTENT OF HAEMOGLOBIN AND RED CORPUSCLES IN THE BLOOD OF MAN AT HIGH ALTITUDES

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That the number of red corpuscles and the content of haemoglobin are increased in the blood of the peripheral capillaries by residence at high altitudes has long been known. In recent years a considerable number of investigators have attempted to determine whether the change is brought about by the mere loss of fluid from the blood without any alteration in the total number of corpuscles or total amount of haemoglobin in the body, or whether the essential thing is an actual new formation of red corpuscles and haemoglobin.

The question at issue has been answered in the affirmative for an active new formation. The problem has been approached in a variety of ways. Thus the observations of Zuntz and coworkers (1) indicated that there was an absolute increase in the amount of haemoglobin and number of red corpuscles, since examinations of stained sections of the bone-marrow from dogs at sea-level and from dogs kept at a high altitude showed for the latter a decrease in fat-cells and an increase in the blood forming elements. This they thought showed an increased activity of the corpuscle producing centers at the high altitudes.

Abderhalden (2) from an extensive series of observations on rabbits and rats, at an altitude of 6,100 feet, concluded that the amount of haemoglobin per animal was not altered, though the amount per kilo body weight as well as the percentage value of the haemoglobin and red corpuscles rose, in that the weight of the animals was uniformly less at the high altitude. He took this to indicate a concentration of the blood without overpro-

duction of haemoglobin. Abderhalden used Welker's method of washing out the blood for the determination of the total haemoglobin and blood volume. Dreyer and Walker (3) have recently gone over Abderhalden's data and found that the blood volume of the rabbits diminished 10.7 per cent at the high altitude, while the haemoglobin readings increased 25.1 per cent. They found that 13.1 per cent of the increase in the haemoglobin was due to a new formation of haemoglobin and that only 12 per cent was due to the diminution in the total blood volume.

Douglas, Haldane, Henderson, and Schneider (4) by the carbon monoxide method of Haldane and Lorrain Smith determined the total amount of haemoglobin and the blood volume of four men during a residence of five weeks on Pike's Peak, altitude 14,109 feet. They found that during the first few days of residence the blood concentrated but that afterwards there was a large increase in the total amount of haemoglobin and a return to, or even a slight increase above, the normal blood volume.

Laquer (5) on Monte Rosa, found that dogs deprived of haemoglobin by hemorrhage of half of their blood supply regenerated it in about 16 days, while at the lower altitudes 27 days were required for the restoration after a similar hemorrhage. These results, like those of Zuntz and co-workers, show the blood forming centers to be more active at high elevations.

Schneider (6) by the carbon monoxide method followed the blood changes in a man who after living six months at an altitude of 14,109 feet returned to a level of 6,000 feet. The total oxygen capacity of the blood decreased gradually in the course of ten weeks 11.9 per cent. This subject destroyed in the period a surplus of 98 grams of haemoglobin. Thus it was shown that there had been an overproduction at the high altitude.

Other recent researches support the evidence that there is an actual increased production of red corpuscles and haemoglobin. Bürker, Jooss, Moll, and Neumann (7) studied the changes in the red corpuscles and the qualitative and quantitative changes in the haemoglobin in four men at Sanatorium Schatzalp, altitude 6100 feet, and found the increase to be an absolute rather than a relative one. Furthermore the blood did not resume its low

altitude composition within a month after they returned to sea-level. Cohnheim and Weber (8) examined the blood of 23 men working on a railway on the Jungfrau Peak in the Alps and concluded that there was no alternative explanation of the increase in corpuscles and haemoglobin save a new formation.

The rise in the number of erythrocytes and the percentage of haemoglobin is especially rapid during the first two to four days of residence at the high altitude, after which there occurs a more gradual increase for about three weeks. Abderhalden's rabbits taken from Basel, 870 feet, to St. Moritz, 6100 feet, showed a percentage increase in haemoglobin of 17.3 the first days. Dreyer and Walker in their review of Abderhalden's data conclude as follows: "But in animals examined within a day or two of their ascent to a high altitude the whole change is due to diminution of the blood volume."

Douglas, Haldane, Henderson, and Schneider are of the opinion that three of their four subjects showed a diminished blood volume during the first days of residence on Pike's Peak. The subject studied most carefully had on the seventh and eighth days about a 15 per cent increase in the haemoglobin and a total blood volume 10.8 per cent less than in Colorado Springs. They concluded that "the increased percentage of haemoglobin on Pike's Peak was apparently due in part, during the first few days, to concentration of the blood."

Evidence that the early increase in erythrocytes and haemoglobin may be due to other factors than the loss of water from the blood is found in a report of experiments by Campbell and Hoagland (9). They carried three rabbits to the summit of Pike's Peak and on arrival counted the number of red corpuscles in the blood taken from the ear and from the mesentery. The average count from the ear in Colorado Springs was 6,087,000 and on Pike's Peak 6,635,000, an average increase of 9 per cent. The average count from the mesentery on Pike's Peak was 5,897,000 or 11 per cent less than the count for the external capillaries at that altitude. They concluded that the early increase in corpuscles was due to a changed vasomotor condition in the peripheral vessels.

BLOOD CHANGES DURING ABDOMINAL MASSAGE

We have recently made two series of observations which shed some light on the rapid increase in haemoglobin and red corpuscles that occurs in the peripheral blood vessels during the first days of residence at a high altitude. In connection with another study (10) in Colorado Springs we had found that massage of the abdomen, or pressure and massaging action obtained by placing on the abdomen a heavy weight of lead foil, weighing about 25 pounds and so shaped as to fit between the lower ribs and the top of the pelvis, invariably increased the amount of haemoglobin and the number of red corpuscles in the peripheral capillaries. This concentration of the blood was caused by the driving onward into the general circulation of a large number of erythrocytes which had been lying dormant in the splanchnic area.

All haemoglobin determinations recorded in this paper were made with the Haldane-Grower haemoglobinometer and the red corpuscles have been counted with the Thoma-Zeiss haemocytometer.

Abdominal massage and pressure experiments on men who had resided some days on Pike's Peak failed to cause the usual increase in the red corpuscles and haemoglobin. Six men served as subjects for the tests. In each experiment the normal content of haemoglobin and number of erythrocytes was first determined in blood taken from a finger. Then with the subject reclining on a bed the abdomen was vigorously kneaded for five or more minutes, after which the weight was placed in such a manner that the respiratory movements continued the massage. In several of the experiments the weight was removed after a time and active massage again given for another period of five minutes. Two determinations of the haemoglobin and red corpuscles were made during each experiment, the first at the end of five or ten minutes and the second after fifteen minutes of massage and pressure. The results appear in Table I. In Colorado Springs a similar treatment of the abdomen raised the haemoglobin content of the blood 3.5 to 7 per cent and increased the number

of red corpuscles 7 to 9 per cent. In only two of the twelve experiments on Pike's Peak was a concentration obtained. In two others a definite change was not evident, while in eight instances the blood was diluted, the content of haemoglobin decreased as much as 1.3 to 3.1 per cent and the number of red corpuscles 0.7 to 5.4 per cent.

The two observations in which concentration occurred find an explanation in the fact that the two men, Atwater and Gregg, had only the day before walked up the Peak and had not at the

TABLE I

Blood changes during massage on Pike's Peak

SUBJECT	DATE	NORMAL		AFTER 15 MINUTES OF MASSAGE			
		Haemoglobin	Erythrocytes	Haemoglobin	Per cent of change	Erythrocytes	Per cent of change
Atwater[1]	June 20	113	6,080,000	118	+4.4	6,560,000	+7.9
	June 23	131	7,197,000	128	−2.3	6,856,000	−4.7
Gregg[1]	June 20	114	6,216,000	122	+7.0	6,776,000	+9.0
	June 22	123	6,744,000	120	−2.6	6,496,000	−3.6
	June 23	121	6,640,000	122	+0.8	6,644,000	+0.1
Havens[2]	June 22	135	7,296,000	132	−2.2	6,904,000	−5.4
	June 26	129		127	−1.6		
Schneider[2]	June 21	120	6,636,000	117	−2.5	6,384,000	−3.8
	June 26	126	6,640,000	124	−1.6	6,592,000	−0.7
Sisco[2]	June 21	132	6,928,000	132	0.0	6,944,000	−0.2
	June 26	126	6,768,000	122	−3.1	6,528,000	−3.5
Robison	June 28	147	7,744,000	145	−1.3	7,504,000	−3.1

[1] Ascended on foot June 19.

[2] Ascended by railway June 16.

time clearly reacted to the influence of the lowered barometric pressure. They arrived at the summit about one o'clock in the afternoon, having climbed four hours. The following morning at eight o'clock Atwater gave a haemoglobin reading of 124 and an erythrocyte count of 6,888,000. This was above his Colorado Springs average of 112 for haemoglobin and 6,224,000 for erythrocytes. Gregg had in Colorado Springs an average of 116 for haemoglobin and 6,514,000 for red corpuscles. Determinations made on him the first morning on Pike's Peak showed practically

no change, haemoglobin 118 and erythrocytes 6,484,000. Neither of the men felt especially well that day and when examined at 4.30 in the afternoon each showed that there had been a decided fall in the haemoglobin and erythrocytes since early morning. It was noted, however, that Atwater's blood was still more concentrated than it had been in Colorado Springs. Gregg's, on the other hand, had fallen below his Colorado Springs average. It was at this time that abdominal massage and pressure caused the marked increase for both men in the content of haemoglobin and erythrocytes in the blood of the peripheral capillaries. Atwater showed as a result of the treatment an increase of 4.4 per cent in haemoglobin and 7.9 in red corpuscles and Gregg had increases of 7 and 9 per cent respectively.

On the following day, June 21, each man made a run, the results of which are given later, so that abdominal massage was not applied again until the 22d. On that day Gregg alone served as subject and then instead of being concentrated the blood was diluted by the massage, the haemoglobin falling 2.6 per cent and the red corpuscles 3.6 per cent. On the 23d Gregg's blood was not as concentrated as on the 22d, hence it is not surprising to find that massage caused a very slight rise in the haemoglobin and red corpuscles. On the 23d Atwater's blood showed the same diluting reaction that massage caused in all the other men we examined on Pike's Peak.

The single observation on Robison, the resident manager of the Summit House, is of more than passing interest in that he had then resided on the summit nearly six weeks. He, too, showed a dilution of 1.3 per cent in haemoglobin and 3.1 per cent in red corpuscles.

These data show that the splanchnic area does not contain in men after the preliminary period of adaptation the large reserve of corpuscles that it holds inactive at the lower altitudes. The diluting effect of massage very likely is caused by forcing lymph from the abdominal viscera into the blood.

THE BLOOD CHANGES DURING PHYSICAL EXERTION

It has been shown by a number of writers (11) that physical exertion at low altitudes causes a pronounced concentration of the blood of the peripheral capillaries. In another paper (10) we have demonstrated that the increase in haemoglobin, in the number of erythrocytes, and in the specific gravity of the blood which occurs during muscular activity results from the sudden passage into the blood of a large number of red corpuscles lying dormant in the body, chiefly in the splanchnic area. In Colorado Springs we have without exception, in the forms of exercise used by us, found the blood to be concentrated during the exertion. The haemoglobin was found to increase 3.5 to 11 per cent and the number of red corpuscles 3.2 to 22 per cent.

On Pike's Peak two forms of exercise similar to those used in Colorado Springs were employed; the first a run on the level of a half and also three quarters of a mile requiring three and a half to five and a half minutes, and second a dash of 175 yards up the "Cog" road track in from thirty-five to forty-five seconds. Six men served as subjects for a total of twelve trials. The results appear in Table II. In eight of these exercise experiments the effort caused no increase in the concentration of the blood, or even resulted in a slight dilution. The first test with Atwater who had walked up was made after he had been on the summit fifty hours. At that time the haemoglobin increased 1.7 per cent, but a similar run two days later when he had reacted well to the altitude failed to concentrate his blood. With Gregg, however, a concentration was obtained after each of the runs made by him. In the first, after a sojourn of fifty hours, the haemoglobin increased 3.5 per cent and the red corpuscles 8.5 per cent. Gregg made his second run forty-eight hours later when he was known to be in better condition. The haemoglobin then increased only 1.6 per cent and the red corpuscles 2.3 per cent. In this connection it is interesting to find that in a sojourn of four days on Pike's Peak Atwater's haemoglobin rose from his Colorado Springs average of 112 to 131, or 17 per cent, and the red corpuscles increased from 6,224,000 to 7,192,000, or 16 per cent. On the other hand

Gregg's haemoglobin on the fourth day had increased from his Colorado Springs average of 116 to 121 only, or 4 per cent, and the erythrocytes had increased from 6,514,000 to 6,640,000, or 1.9 per cent.

The above observations suggest that as a person reacts to the influence of the high altitude the reserve corpuscles are thrown into the general circulation. As a consequence there is in individuals who have reacted well to the lowered barometric pressure no reserve to bring out during strenuous physical exertion and for

TABLE II

Blood changes during exercise on Pike's Peak

PERSON	DATE	NORMAL		AFTER THE RUN	
		Haemoglobin	Erythrocytes	Haemoglobin	Erythrocytes
		Ran from 0.5 to 0.75 mile			
Atwater...	June 21, '14	118	6,392,000	120	6,432,000
	June 23, '14	133	6,984,000	133	6,912,000
Gregg.....	June 21, '14	115	6,100,000	119	6,616,000
	June 23, '14	122	6,640,000	124	6,792,000
Havens....	June 2, '13	126	6,900,000	126	6,900,000
	June 27, '14	129	7,048,000	130	7,104,000
Sisco.....	June 1, '13	120	6,900,000	120	6,800,000
	June 27, '14	128	6,736,000	129	6,712,000
		175 yards up the "Cog" track			
Eager.....	Oct. 26, '13	115	6,472,000	114	6,238,000
Havens....	June 28, '14	129		126	
Robison...	Oct. 27, '13	150	8,256,000	152	8,376,000
Sisco.....	June 28, '13	134		133	

this reason the blood does not concentrate during exercise at the high altitude as it regularly does at lower altitudes.

It is unfortunate that we did not have Gregg run on the 22d when it was found that abdominal massage diluted the blood. On the 23d an abdominal massage experiment made two hours before the run raised the haemoglobin 0.8 per cent and slightly increased the number of red corpuscles. The fact that such strenuous exertion as a half mile run increased the haemoglobin only 1.6 per cent makes it probable that longer continued and more vigorous massage would, very likely, have raised the haemo-

globin content an equal amount. We believe that these experiments indicate that the splanchnic area is the chief reserve of red corpuscles.

Robison, the resident manager of the Summit House, kindly ran for us a distance of 175 yards up the steep grade of the "Cog" road on October 27, 1913. He had then resided on the summit of the Peak over five and a half months. This run increased his haemoglobin 1.3 per cent and the red corpuscles 1.4 per cent. The same autumn ten days after he had descended to Colorado Springs he ran again in one of the college buildings doing about the same amount of work in stair climbing. His haemoglobin then increased from 142 to 149, or 4.9 per cent, and the erythrocytes from 6.8 to 7.4 millions, or 8.8 per cent. Our abdominal massage experiment was made on him the following summer when he had been on the summit less than six weeks. It was impossible to try a work experiment at that time. These observations on Robison suggest that even in those acclimatized to very high altitudes there is not a large reserve supply of corpuscles available to meet the increased demand for oxygen during exertion.

Laquer (5) reported that after hard muscular work there was a decrease in the concentration of the blood of the fingers at the high altitude, this he concluded was the result of a vasomotor change. We found on Pike's Peak that the content of haemoglobin and erythrocytes immediately at the end of exercise was usually the same as before exercise. Later, however, the blood slowly diluted just as it does at lower altitudes. Hence within a few minutes the concentration will show a decrease. Often this diluting process was more marked than in Colorado Springs.

Our two series of observations on the influence of abdominal massage and pressure, and of muscular exertion on the blood changes show that a part of the rise in the number of erythrocytes and in the percentage of haemoglobin that occurs during the first two to four days of residence at high altitudes is caused by throwing into the general circulation a large number of corpuscles that have been inactive in the splanchnic area, and very likely to some extent elsewhere, at lower altitudes. The stimulus that calls forth these resting cells is quite likely associated with the

demand of the tissues for oxygen. At high altitudes a need for oxygen is felt even during rest. That there is a shortage of oxygen during the first days of residence has been clearly shown by Douglas, Haldane, Henderson, and Schneider.(4). All available corpuscles are soon thrown into the general circulation in the effort to supply this need, with the result that during muscular work there are no corpuscles remaining in reserve, consequently there is no increase with exercise.

The changes in the blood on adaptation to high altitudes may here be briefly summarized. A rapid increase in the number of red corpuscles and percentage of haemoglobin in the blood of the peripheral vessels occurs during the first two to four days of residence at the high altitude, then follows a more gradual increase for about three weeks. The initial rapid increase is brought about in part by throwing into the systemic circulation a large number of red corpuscles that under ordinary circumstances at low altitudes are side-tracked and inactive; and in part by a concentration resulting from a loss of fluid from the blood. The more gradual increase in red corpuscles and haemoglobin extending over several weeks is brought about by the increased activity of the blood forming centers so that there results a large increase in the total number of corpuscles and amount of haemoglobin.

THE SEQUENCE OF BLOOD CHANGES DURING THE EARLY DAYS OF RESIDENCE ON PIKE'S PEAK

The rise in the haemoglobin content of the blood has been followed in three subjects during three to five expeditions to the summit of Pike's Peak. In these several expeditions the rate of increase of the haemoglobin has varied. We believe the differences observed find an explanation in the condition of each subject at the time of the journey. Five men were induced to walk up the mountain so that it was possible to determine the effects of fatigue when added to the influence of lowered barometric pressure. Among our subjects there was opportunity to compare the influence of altitude on men in excellent physical condition because of regular training in muscular activity and on men who

had led an inactive or sedentary life. In three of our expeditions only the change in the haemoglobin content of the blood was recorded while in the fourth the number of red corpuscles was also counted. These data are given in Tables III, IV and V.

TABLE III

(*Haemoglobin*)

DATE	TIME	HAVENS	SCHNEIDER	SISCO	
Average.....		110	110		Colo. Springs
Oct. 11, 1912.....	12 noon	110	110		Pike's Peak, just arrived
Oct. 12.....	10 a.m.	118	117		
Oct. 13.....	8.30 a.m.	127	124		
Oct. 14.....	9.30 a.m.	129	118		
Average.....		116	109	113	Colo. Springs
May 29, 1913.....	7 p.m.	116	109	112	Pike's Peak 7 hrs. after arrival
May 30.....	6 a.m.	124	110	118	
May 31.....	7 a.m.	128	113	121	
June 1.....	7 a.m.	130	114	123	
June 2.....	7 a.m.	129	115	121	
June 3.....	7 a.m.	134			
Average.....		111	110	111	Colo. Springs
Oct. 24, 1913.....	7.30 a.m.	113	113	116	Pike's Peak,[1] 1st morning
Oct. 25.....	7.30 a.m.	114	112	115	
Oct. 26.....	8 a.m.	115	115	115	
Oct. 27.....	7 a.m.	119	116	118	
Average.....		109	109	113	Colo. Springs
June 17, 1914.....	7.30 a.m.	123	116	120	Pike's Peak, 1st morning
June 18.....	8 a.m.	126	115	125	
June 19.....	7 a.m.	129	122	126	
June 20.....	8 a.m.	130	121	122	
June 21.....	8.30 a.m.	132	123	130	
June 22.....	8.30 a.m.	135	121	133	
June 24.....	8 a.m.	135	126	134	
June 26.....	8.30 a.m.	134	127	131	
June 28.....	7 a.m.	129	129	135	
June 29.....	8 a.m.	132	129		

[1] Havens walked up the Peak October 23, 1913.

All of the subjects of the first two expeditions ascended the mountain in the "Cog" railway train. The haemoglobin content of their blood was determined within a few hours after arrival

and in each subject it was found that the haemoglobin was not altered immediately by entrance into rarefied air. However, in all subjects who had reacted well to the influence of oxygen want a well defined increase in haemoglobin was found to have occurred by the morning after that of the ascent.

Havens has been under observation during four trips to the summit of Pike's Peak (see Table III). Three times the ascent was made by railway car and the fourth he walked up the mountain. Following the three times of passive ascent the increase of haemoglobin was rapid: thus the morning after the ascent in October 1912 it was up eight points by the Haldane-Gower haemoglobinometer, or 7.3 per cent; in May 1913 also eight points, or 6.9 per cent; and in June 1914 fourteen points, or 12.8 per cent. Before each of these three trips Havens had been muscularly active. Prior to the first, in 1912, he had worked out-of-doors all summer at manual labor. In May 1913 he was in perfect training, having exercised daily for three months in preparation for the two mile run in intercollegiate contests. It is interesting to find then that his average for haemoglobin in Colorado Springs was 116 or six points higher than at the time of the first expedition and yet beginning at this higher level the haemoglobin had increased an equal number of points by the first morning after the ascent. In the spring of 1914 Havens again trained for the two mile run for a time but discontinued regular running about three and a half weeks before the trip to Pike's Peak. His normal haemoglobin content dropped during this interval from about 115 to an average of 109. It is interesting to find, therefore, that in this expedition the increase in haemoglobin on the first morning was greater than in the earlier expeditions, having risen from the average lower level of 109 to 123 in about twenty hours. This increase is equal to the sum of the increases obtained the year before by athletic training and altitude influence. In each of these expeditions his haemoglobin reached practically the same level by the third morning of residence on the Peak; these were 129, 130, and 129 respectively. During our residence of two weeks on the Peak in 1914, the haemoglobin reached its maximum—135—in six days. In 1913 it was 134 on the fifth morning.

In the autumn of 1913 Havens made the ascent on foot. While he was not in such splendid physical condition as the June prior he was nevertheless physically strong. However, apparently as a result of the fatigue of the climb, his haemoglobin had increased only 1.8 per cent the morning after the ascent. Furthermore, the rate of increase was less on the following days than it was in the other expeditions. Thus on the third morning the haemoglobin content was 115 instead of 129, the average reading for that day on the other expeditions. On the fourth morning it had risen only 7 per cent above the Colorado Springs average instead of 12 to 19 per cent as it had during the other trips.

Five series of observations on Schneider are available, the four recorded here and a series with the English-American Pike's Peak expedition (4). It is interesting to find that in three of these sojourns Schneider was quite ill with mountain sickness and that during the other two he was not greatly disturbed by the altitude. In the English-American expedition his haemoglobin was up only two per cent the first morning. It increased in the same time in October 1912, 6.4 per cent, May 1913 only 0.9 per cent, October 1913, 2.7 per cent, and in June 1914, 6.4 per cent. The small rises noted were associated with the three periods of rather severe mountain sickness. Prior to the trip in October 1912 when the haemoglobin increased rapidly Schneider had spent the summer in out-door activities involving considerable physical exertion. Before the trip in June, 1914, at which time the haemoglobin also rose quickly, he had taken regular tramps in the mountains and played tennis several times a week. Before the other excursions Schneider had not taken regular exercise. It appears then that the rate of change in the haemoglobin during the first twenty-four hours spent at high altitudes depends upon the physical condition of the subject.

The rate of change for Schneider also varied in a similar manner on the subsequent days. Thus for the two trips when he was in excellent physical condition the haemoglobin increased to 124 in two days in October 1912 and to 122 in three days in June 1914. At the time of the English-American Pike's Peak expedition he had just returned from sea-level and his haemoglobin in Colorado

Springs was only 101. Starting from this low level the haemoglobin increased slowly to 122 during the first fourteen days spent on Pike's Peak. The maximum for this trip was 123. In June 1914 the maximum of 129 was reached in twelve days. The maximum obtained in each of the remaining expeditions in four days was only 115 and 116 respectively. These figures are far below those obtained in the expeditions when he was physically strong.

Sisco participated in three expeditions. He never had taken regular exercise, he may be regarded as representing the semi-sedentary type of subject. His haemoglobin increased during the first twenty hours of residence on the summit 4.4, 4.5, and 6.2 per cent respectively in the three sojourns. Sisco had at the time of the ascent in October 1913 a slight bronchitis and suffered some from headaches which may account for the fact that his haemoglobin increased less rapidly this stay than during the other two. During his first trip, June 1913, the haemoglobin increased steadily for three days to 123 and in June 1914 it reached 126 in the same time. In October 1913, however, when he was not well, on the third morning it was no higher than the first morning when it was 116.

In addition to Havens we have had four more subjects ascend Pike's Peak on foot (see Table IV). Eager and Munro walked up with Havens in October 1913. These men were chosen because it was thought that they represented three conditions of physical fitness. Havens, although not in as splendid condition as the June preceding, had exercised the most; Munro and Eager had exercised very little, but Munro seemed the more fit of the two. It is interesting, therefore, to find on the first morning after the ascent that Havens' haemoglobin was up 1.8 per cent, that Eager's had not changed, while Munro's was 2.7 per cent below his Colorado Springs average. In four days Havens' haemoglobin increased 7.2 per cent, Munro's 1.8 per cent, and Eager's had not clearly altered. Havens felt very well throughout the stay on the summit, Eager and Munro could not sleep the first night and suffered more or less with headache throughout their stay.

The morning of June 19, 1914 Atwater and Gregg walked up the Peak and remained on the summit until the evening of the 23d. Atwater reacted well, the morning following the ascent his haemoglobin and red corpuscles were each up 10.7 per cent. That day a headache developed and by afternoon his haemoglobin had decreased from 124 to 113, the red corpuscles from 6,888,000 to 6,080,000. By the next morning both elements had again made a material gain but had not reached the level of the first morning. On the fourth and last morning Atwater's haemoglobin was 17 per cent above his Colorado Springs average and the red corpuscles up 15.5 per cent. He reacted to the influence of the high altitude even better than Havens had after

TABLE IV

Haemoglobin of men who walked up the Peak

MORNING	ATWATER[2]	GREGG[2]	EAGER[1]	MUNRO[1]	
Average	112	116	112	113	Colo. Springs
1	124	118	112	110	Pike's Peak
2	120	117	114	113	
3	122	123	115	114	
4	131	121	113	115	

[1] Walked up October 23, 1913.
[2] Walked up June 19, 1914.

his climb in the October trip. Atwater had for ten or more days prior to his trip been working regularly out-of-doors as a house painter. It was his opinion that he was in most excellent physical condition.

The morning after the ascent Gregg showed an increase of only 1.7 per cent in haemoglobin and no definite change in the number of red corpuscles. He suffered from mountain sickness the first night on the Peak and had a headache the first two days. Gregg had not followed a system of regular exercise and had led only a fairly active life before joining the expedition. In four days his haemoglobin increased only 4.3 per cent and the red corpuscles only 1.9 per cent. His increase in corpuscles throughout the stay was uniformly less than that of the haemoglobin.

The red corpuscles were counted only in the expedition of June 1914. It will be observed, however, that they and the haemoglobin, in all subjects except Gregg, varied together and about equally. These counts appear in Table V.

The data obtained in our study of the changes in the haemoglobin and red corpuscles in these several expeditions show that both, in physically fit persons who ascend the mountain passively, increase rapidly within twenty-four hours. This reaction does not begin immediately on entrance into the rarefied air. There

TABLE V

(*Red corpuscles per cubic millimetre*)

DATE	HAVENS	SCHNEIDER	SISCO	ATWATER	GREGG	
Average.....	6,024,000	5,992,000	6,372,000	6,224,000	6,514,000	Colo. Springs
June 17, '14.....	6,872,000	6,472,000	6,732,000			Pike's Peak, 1st morning
18.....	7,024,000	6,400,000	6,880,000			
19.....	7,160,000	6,800,000	6,720,000			
20.....	7,292,000	6,848,000	6,624,000	6,888,000	6,484,000	
21.....	7,200,000	6,736,000	6,928,000	6,512,000	6,304,000	
22.....	7,296,000	6,472,000	7,032,000	6,656,000	6,744,000	
23.....				7,192,000	6,640,000	
24.....	7,248,000	6,616,000	7,104,000			
26.....	7,000,000	6,656,000	6,856,000			
28.....	6,840,000	6,896,000	7,120,000			
29.....	6,976,000	6,960,000				

then follows a period of several days, two or three, in which the concentration although somewhat reduced is still quite rapid, after this there may be for a time a continued slow increase. In subjects known to be in poorer physical condition these blood changes occur more gradually and in such subjects symptoms of mountain sickness may appear.

Among subjects who ascend to the high altitude on foot the value of physical fitness or training is very evident. Men in excellent condition react quickly but not, as observations on Havens indicate, so decidedly as when they ascend passively by train. Men not accustomed to strenuous exertion may not react at all the first twenty-four hours after the ascent and only slowly thereafter. We believe one value of physical training is

to be found in the ability of the organism to throw into the general circulation when there is an increased demand for oxygen a large number of red corpuscles that have been side tracked, so to speak, and, therefore, inactive. This throwing of corpuscles into the general circulation accounts in large measure for the first rapid rise in haemoglobin and erythrocytes.

It is the opinion of Dreyer and Walker (3) that the change of blood volume which occurs with a particular change of barometric pressure is proportional to the area of the body surface of the individual. They also believe that the whole increase in haemoglobin and red corpuscles observed within the first day or two after ascent to the high altitude is due to a diminution of the blood volume. If their reasoning is correct the concentration of the blood for a given subject should be about the same each time he goes to a particular altitude. We have shown, however, that in five journeys to the summit of Pike's Peak the haemoglobin content of Schneider's blood had increased in twenty hours as little as 0.9 per cent and as much as 6.4 per cent. Havens and Sisco who also have been with several expeditions have shown similar variations. Dreyer and Walker's explanation fails to account for these variations, while they find an explanation in the theory advanced above.

SUMMARY

1. At low altitudes abdominal massage increases the number of red corpuscles and percentage of haemoglobin in the peripheral capillaries. In men partially or wholly acclimatized to a high altitude abdominal massage lowers the content of haemoglobin and red corpuscles. Before the subject reacts to the influence of lowered barometric pressure, abdominal massage may raise the content of haemoglobin and red corpuscles.

2. Physical exertion at low altitudes concentrates the blood but during a sojourn of four days to two weeks at a high altitude this reaction occurs only in the period before the number of red corpuscles and percentages of haemoglobin have increased. In a subject who had lived five and a half months at 14,109 feet a given exercise caused a slight concentration of the blood but not as much as it did at the altitude of Colorado Springs.

3. While there is a reserve supply of corpuscles at low altitudes this is lacking for some time during residence at the high altitude.

4. The number of red corpuscles and percentage of haemoglobin do not increase immediately on arrival at the high altitude. Usually there occurs within twenty-four hours a marked increase in both.

5. The rise in haemoglobin and red corpuscles for a particular subject during the first three or four days spent at a high altitude is not the same for different visits. The increase is most rapid in subjects who have taken regular exercise before ascending to the high altitude.

6. Fatigue due to walking up a mountain delays the altitude increase in haemoglobin and red corpuscles.

7. The rapid increase in the number of red corpuscles and percentage of haemoglobin the first two or three days spent at a high altitude is due in part to the throwing into the general circulation of a large mass of reserve corpuscles, and in part to a loss of fluid from the blood. The blood forming centers also become more active and increase the total number of corpuscles and total amount of haemoglobin.

We wish here to express our hearty appreciation of the twelve Colorado Springs friends who generously supported our expedition in June 1914, and to the young men who so kindly served as subjects.

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THE INFLUENCE OF LIGHT ON REPRODUCTION IN VORTICELLA

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The aim of the work briefly described in the article, has been to ascertain the influence of monochromatic, and different intensities of light on the reproduction of vorticella.

The vorticella were transferred by means of a Barber[1] capillary pipette, held in a modified triple movement pipette holder.

From a culture of the unicellular organisms, that had been kept covered in the dark, for several months, many encysted zooids were isolated, and transferred to a culture media, consisting of an infusion of Timothy hay. The infusion had been filtered, sterilized by boiling, and kept in sterilized sealed tubes until needed.

The encysted forms were under observation in a hanging drop placed in an open end moist chamber.

As soon as the zooids emerged from their cysts, they were removed to a drop of the sterilized infusion placed on a Hemocytometer micrometer slide. By this means, the direct increase in organisms could be ascertained. The slides with the young zooid were placed in a Petri-dish that contained a thin layer of water to prevent evaporation of the solution. The organisms of the same age thus secured, were kept for each series of observations, under the same conditions of temperature, moisture, food and intensity of light.

To ascertain the effect of colored rays, blue, green, yellow and red glass, gelatin films, and reflected rays from Hering's monochromatic colored cards were employed. The observations thus

[1] Barber M. A.: Jour. of Infections Dis., 1911, Vol. viii, p. 348.

secured were compared with those made in sun or electric light of constant intensity; the colors were placed both in bright and dim light and so that the reflected or transmitted rays penetrated the culture either from above or below. The heat rays were cut out by water contained in parallel sided glass dishes placed in the path of the beams of light.

In order to study the influence of the intensity of light, the slides with the vorticella were placed either half or one and a half or two meters from a window having southern exposure or electric light. Some were placed in a large blackened wall box or in a box covered with smoked glass to vary the intensity of the rays of sunlight.

From the observations that were obtained from the various experiments the following general conclusions were deduced.

1. Vorticella exposed to daylight for intervals of four days increase in number more rapidly on bright sunny days than on cloudy dark ones.

2. When the zooids are placed at different distances from the source of light on an average 25 develop one half meter, ten one and one half meter from the light and two in the dark.

3. In comparing the greatest number that developed on sunny days from one zooid in 24 hours it was seen that 40 grew under green, 29 under yellow, 26 under white and 13 under blue, 17 under red.

4. The average rate of increase on cloudy days or under dim light was 4 for yellow, 3 for green, 2 for blue, white or red.

5. We conclude that the stimulating effect on the reproductive power of vorticella increases up to an optimum with increased intensity of light, and that the bright luminous rays of yellow and green are more effective as stimulating agencies on the reproductive power of vorticella than are the red or blue rays.

EXPERIMENTS ON X-RADIATION AS THE CAUSE OF PERMEABILITY CHANGES

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Studies on the effect of radiation by Roentgen rays upon eggs have shown that these rays are capable of causing changes in the activities of the egg and of certain of its parts. Among others, changes in the rate of cell division, differences in the behavior of chromatin in division, and variations in the activity of cell extracts have been noted as the result of exposure to these rays. Some, at least, of the departures from normal are similar in character to the results obtained by applying various experimental methods to living eggs. It has been suggested therefore that changes in the permeability of the plasma membranes of the egg cells may be the basis for the abnormal conditions observed. This suggestion comes from the fact that changes of such nature are held to be responsible for cytolysis of the cortical layer of the egg substance, and the processes which, as initiation of cell division according to Loeb, are consequent to cytolysis; for metabolic changes such as the increase in the elimination of CO_2 and of catalase and increase in oxygen absorption; and for various other physiological reactions of the cell.

There would seem to be a number of a priori reasons for expecting permeability changes to be brought about by radiation. McClendon found evidence for the increase in permeability of the sea urchin's egg at the beginning of development, and he states that "there is some indirect evidence that increase in permeability may cause an increased division rate in tissue cells. Though cell growth may influence division, it is probable that permeability influences growth." My observations that expos-

sure of Planorbis eggs to X-rays hastens the rate of division, could be most easily explained, perhaps, on the basis of this assumption. McClendon also cites the case of cancers that have been produced by the action of X-rays upon the skin. "The cells in the skin were changed so that they proliferate more rapidly. Similarly, electrical changes have been observed to start the egg cell to rapid proliferation" (tissue cells have also been electrically stimulated to increased division). He thinks that "there is some irreversible change in the permeability of these cells." It is, furthermore, to be expected that perhaps an agent which brings about the profound internal disturbances known to be caused in the chromatin by radiation, as shown by the researches of the Hertwigs and others, would work extensive changes at the surface where it first comes into relation with the cell, namely, at the plasma membrane.

If permeability changes are caused by radiation, then the general results of that treatment are probably to be brought in line with effects produced by other experimental methods. On the other hand, if no permeability changes are demonstrable it is necessary to look more deeply for the causes underlying the disturbances and it becomes increasingly probable that the effects of radiation are specific, or at least dependent upon some more fundamental principle for their explanation.

Thus the investigation of the question, whether changes in the permeability of the plasma membrane can be brought about by X-radiation, becomes a matter of some theoretical importance. The conclusion reached here rests solely upon the evidence available from experiment.

Four methods for investigating this question were available to the writer. (1) All agents which cause permeability changes cause an exudation of pigment from the integument of Arenicola larvae. (2) The prevailing hypothesis is that the initiation of all division which is the first step in artificial parthenogenesis is due to changes in the permeability of the plasma membrane to cytolysing agents. (3) The indicator method depends upon the sensitivity of neutral red, the indicator, for detecting the presence of alkali, in this case, NaOH, in the cell. (4, an adaptation

of 3). Permeability changes are indicated in certain plant cells where, by the use of an indicator (neutral red in Elodea as suggested by Harvey, '10), the change in content of the cell can be detected when placed in a solution to which the membrane is permeable.

Previous experiences with the physiological applications of X-rays had shown that the effective strengths under the conditions which obtained with my experiments were a short accelerative radiation of two or three minutes on the average, a non-effective one of about five or six minutes, and a longer inhibitive radiation. I have not been able to observe much difference in effect between ten, fifteen and thirty minute radiations, so for most of the work only second of these stronger radiations was used. Apparatus and current were not available for exposures of great intensity or longer duration.

Arenicola larvae. R. S. Lillie has pointed out the usefulness of Arenicola larvae for indicating permeability changes. When these small trochophores "are suddenly brought from sea water into pure isotonic solutions of sodium salts the muscles contract with extreme vigor and persistence, causing the larvae to shorten to half their normal length; at the same time the yellow pigment contained in the cells of the organism diffuse into the solution and colors the latter yellow. The exit of the pigment is the impression of a rapid permeability-increasing or cytolytic action." I have radiated larvae of Arenicola for varying lengths of time (two, five and fifteen minutes at a distance of about four inches from the tube) and have observed them immediately after the radiation as well as at intervals up to an hour following it. No muscular contractions were caused by the radiations. (Increase in permeability here is usually associated with acceleration of movement.) The larvae were injured in no way so far as could be observed; and no pigment diffused out into the water even on the light side of the drop where the larvae were most densely massed due to their strong phototropism. These experiments were repeated and in no case were results obtained different from that just stated. The general conclusion is to be drawn therefore, that radiation does not cause changes in the permeability of these larvae, or that any changes so caused are too slight to be detected by this method.

Artificial parthenogenesis. On the assumption that a substance must enter the egg in order to affect it (that is, the permeability of the plasma membrane must be changed) Loeb has suggested as a test of permeability change the effectiveness of a substance in solution in causing artificial parthenogenesis. Artificial parthenogenesis is held by him to be due to the formation of the fertilization membrane which in its turn is caused by cytolysis of the cortical layer of the egg protoplasm. Cytolysis would imply, therefore, a change in permeability for its occurrence. If not positively proven, this theory without doubt serves excellently as a working hypothesis and it is allowable to proceed on that assumption. Experiments looking toward the production of artificial parthenogenesis by radiation are, therefore, of interest in this connection.

Bohn reported that the eggs of the sea urchin were more easily fecundated after exposure to radium rays although the sperms were rapidly killed. He also records having obtained parthenogenesis. "Si enfin on expose des oeufs non fécondés aux rayons du radium, certains de ces oeufs (2 à 4 pour 100) évoluent sans le concours d'un spermatozoide (parthenogénèse); on obtient des embryons irréguliers, en general des demimorula de 4 ou 8 cellules attachées à un gros blastomère subissant rarement une bipartition." The small percentage of embryos obtained and the slight extent of their development render these results of doubtful significance. The percentage of parthenogenetic embryos of sea urchins now artificially obtained by other agencies are so large that this case should probably be rejected at least until it has been verified by new experiments.

The incomplete parthenogenesis described by the Hertwigs as the result of radiation by radium does not come into consideration here, for by parthenogenesis they mean only the development of the embryo without the participation of the sperm nucleus. The sperm actually entered the egg but was prevented from taking part in subsequent development by the radiation. But it was able to initiate development before this destructive effect occurred, and it is the initiation of development with which we are here concerned.

The writer performed a number of experiments to see whether X-radiation could be used as a means of inducing development by artificial parthogenesis. Loeb's "improved method of artificial parthenogenesis for sea urchins (Artificial Parthenogenesis and Fertilization, p. 71) consists of two parts. The first is the treatment with the parthenogenetic agent, e.g., 50 cc. sea water + 2 cc. $\frac{N}{16}$ butyric acid for one and one-half to three minutes; the eggs are then transferred to sea water for about a quarter of an hour. Then the "corrective," the second part of the treatment was given by transferring the eggs to hypertonic sea water (50 cc. sea water + 8 cc. $2\frac{1}{2}$ M NaCl) for twenty to twenty-five minutes. There are, of course, many other methods of causing artificial parthenogenesis, but as this method is well worked out and has become a standard, which is almost certain to give results, only it and modifications of it were tried for the experiments here described. Perhaps other methods might have given results of different character but for various reasons it was not thought feasible to try them. Attempts were made to use radiation in place of both the first and second treatments just described and also in connection with certain parthenogenetic agents to accelerate or retard them actively. The following experiments are selected from the entire number performed as examples.

EXPERIMENTS UPON NEREIS EGGS

July 23, 3.15 p.m. Radiated Nereis eggs two minutes (Labelled N. 2), kept part of N. 2 for control. It was not further treated. No jelly nor polar body had been formed by 10 p.m. next day.

3.34. Put second part, N. 2. a, in sperm suspension (fertilized with sperm normally).

4.50. N.2.a shows beginning of cleavage furrow.

5.05. Cleavage furrow nearly complete.

6.00. Second cleavage.

July 24. 10 a.m. 95 per cent normal ciliated embryos.

July 23 3.34. Put third part, N.2.b, in KCl $\frac{M}{4}$ for twenty minutes. Then divided it into two lots.

3.54. One lot, N. 2. b sp., was fertilized with sperm suspension.

4.38. N. 2. b sp. showed first polar body.

July 24. 10 a.m. Cytoplasmic masses somewhat irregular in character. No development.

July 23. 3.54. Second lot of N. 2. b placed in sea water, labeled N. 2. b. w.

4.38. N. 2. b. w. showed first polar body.

July 24, 10 a.m. Cytoplasmic masses irregular in shape; show "segregation." No parthenogenesis.

July 23. At this same time another series of experiments of the same character were performed upon other eggs of the same lot. These eggs however were radiated fifteen minutes, beginning at 3.15 p.m. (N. 15)

Kept part of N. 15 for control. It was not further treated.

July 24. 10 a.m. No jelly or polar body had been formed.

3.35. Put part, N. 15 a, into sperm suspension, normal fertilization.

4.50. N. 15 a. shows beginning of cleavage furrow.

5.05. Cleavage nearly complete.

6.00. Four-cell stage.

July 24. 10 a.m. 99 per cent normal ciliated embryos.

July 23. 3.35. Put part of N. 15 into KCl $\frac{M}{4}$ for twenty minutes, N. 15 b. These eggs were divided into two lots, one of which (N. 15. b. sp.)

3.55. Was fertilized with sperm suspension.

4.38 N. 15. b. sp. gave off first polar body.

July 24. 10 a.m. Cytoplasmic masses, slightly irregular; they show little segregation, but except for polar bodies give no evidence of development.

3.55. Second lot of N. 15. b. were placed in sea water. N. 15. b. w. No further development except polar body formation, as in N. 15. b. sp.

At the time when these experiments were set up, some normal unradiated eggs were fertilized with unradiated sperm. These (July 24, 10 a.m.) were all alive, were swimming more rapidly, and were in a healthier condition than any of the experiment, even N. 2. a. and N. 15. a. Samples of these as well as of N. 2. a. and N. 15. a. were fixed.

Thus the radiation did not prove effective as a means of starting development in these experiments.

To secure parthenogenetic development in Nereis, the first step is to cause the formation of the egg jelly. There are various methods of accomplishing this, but the one used in a set of experiments to be described here in which jelly formation was brought

about before radiation of the eggs, is to be credited, I believe, to E. E. Just (whose work is as yet unpublished).

The eggs were heated to 35 degrees in water for five minutes. This caused the jelly to form. The eggs were then radiated, part for two minutes and part for fifteen minutes. This treatment ended at 3.31 p.m. At the end of the radiation irregular membranes were to be seen about the eggs. The germinal vesicles did not show. When observed at 3.55 the first polar body had been formed in both and five minutes later the second was given off.

At 6.00 p.m. "segregation" had begun. Some had divided irregularly once and then begun segregation. The same conditions were met with in both radiations.

Some of these eggs had been mixed with sperm after radiation but as the jelly had been formed the sperm were unable to enter and development had not followed. At 6.00 p.m. these, too showed segregation.

The experiments given are typical for Nereis. I was never able to secure results fundamentally different from the above. They point to the conclusion that X-radiation is not an effective means of producing artificial parthenogenesis either in a first or second treatment for Nereis.

[*Note.* In this connection it may be well to call attention to the commonly observed fact that Nereis eggs when first shed are very irregular in shape, owing doubtless to their crowded condition in the animal's body. In the sea water they round up and finally take on the characteristic shape. The phenomena shown here and those concerned with the cortical changes subsequent to fertilization indicate that the membrane is "permeable for both crystalloids and colloids at this time." (Lillie, F. R.) If freshly shed eggs be radiated (whether immediately fertilized or not) and carefully observed in comparison with non-radiated eggs it will be seen that the changes in shape occur at exactly the same time in the two lots. If any change in permeability resulted from the radiation it could scarcely be that both lots should round up at once, or as is the observed fact that the jelly in the fertilized eggs should be extruded in both radiated and

unradiated eggs simultaneously. These observations have been repeatedly made and are in line with the evidence from other experiments that radiation does not cause permeability changes.]

EXPERIMENTS ON ARBACIA

July 29. 9.06. Arbacia eggs were radiated for two minutes. (A 2.)

9.11. Others of same lot were radiated for fifteen minutes. (A 15).

9.33. Both lots were treated with hypertonic sea water (50 parts sea water plus 8 parts $2\frac{1}{2}$ M. NaCl) for twenty minutes and then transferred to sea-water.

10.00. When observed at this time a few eggs in each lot had given off polar bodies.

11.00. A 15. Membranes not formed. But one case of cleavage was observed.

2.30. A 2. Six eggs of the entire lot were observed to have divided irregularly.

A 15. Five eggs of the entire lot had divided.

July 30. A 2. Around the outside of the dish there were perhaps $1\frac{1}{2}$ per cent of swimming blastulae; in the center of the dish where the eggs were somewhat more numerous there were no swimming embryos. Of the whole lot not more than 2 per cent were swimming. Through the dish there were a few fused masses which had lost their pigment but could swim about slowly. Membranes had not been formed on the eggs which did not divide.

A 15. Only one swimming blastula observed in dish which contained quite a large number of eggs. There were no fusions in this dish.

July 31. All had disintegrated.

2 p.m. Some were left standing untreated for a control.

August 2. 3 p.m. About 10 per cent had divided once or twice and disintegrated. No membranes were formed.

This small number of living embryos might be explained as the result of several things. It might be thought that a few sperm in the dish caused the development of these eggs, but the most careful precautions were taken to prevent introduction of sperm and the writer cannot believe that these few eggs were fertilized with sperm. The length of time elapsing before division is what would be expected for parthenogenetically produced larvae rather than after normal fertilization. It is also possible

that these larvae are to be explained, as in the case recorded by Bohn, as the result of radiation. But Loeb has shown that hypertonic sea water itself, without further treatment will cause development in some instances. I have also found a few cases of division in other experiments caused by this treatment alone. In view of this fact and because of the small number occurring in the dishes, it is not justifiable to conclude that radiation will serve as the first factor in causing parthenogenetic development.

Other experiments were performed to find whether radiation could be made to serve as a second factor. On August 1 a series of experiments was set up from the same lot of eggs.

August 1. 2 p.m. Some were treated with hypertonic sea water for twenty minutes and then transferred to sea water.

August 2. 3 p.m. No membranes had been formed. About 5 per cent had divided once or twice irregularly and disintegrated.

August 1. 2 p.m. Other eggs were divided into two lots after having been treated for twenty minutes with hypertonic sea water.

One part (A 2) was radiated for two minutes, and the other (A 15) for fifteen minutes.

August 2. 3 p.m. In both A 2 and A 15 fewer of the eggs had disintegrated than in the other experiments of this set, just mentioned. A few irregular divisions had occurred. No embryos, living or dead, were present in the dishes.

Some of these eggs were radiated two minutes. Half were then treated with the hypertonic sea water. On the next day the following observations were made: half-disintegrated, no membranes, some three-celled eggs, no blastulae.

The other half were radiated but not given any subsequent treatment. No blastula had developed next day and there was a general disintegration of the eggs.

Similarly, some eggs were radiated for fifteen minutes. There were only a few irregular divisions next day and most of the eggs in both parts of this lot (those treated and those not treated with hypertonic sea water) were disintegrated.

It is unnecessary to give further experiments. These will serve to show that the writer was unable to produce artificial parthenogenesis in sea-urchin eggs.

EXPERIMENTS WITH ASTERIAS

It is in general easier to produce artificial parthenogenesis in star fish eggs than in those of the sea-urchin, and Loeb's finding should be noted, that "the starfish eggs differ from those of the sea-urchin in this, that they do not depend upon the second corrective factor with the same degree of necessity." Hence, attention was turned to this form, for if there is any hope of producing artificial parthenogenesis with radiation that result should be obtained here.

Eggs were taken from a starfish and divided into lots, these lots were treated as follows:

1. Eggs were left without treatment of any kind.
2. Eggs were radiated for two minutes, then seventeen minutes later were treated with hypertonic sea water for twenty minutes.
3. Eggs were radiated two minutes. No subsequent treatment.
4. Eggs were radiated fifteen minutes, then treated for twenty minutes with hypertonic sea water.
5. Eggs were radiated fifteen minutes. No subsequent treatment.
6. Eggs were treated twenty minutes with hypertonic sea water. They were not radiated.
7. Eggs were treated as in 6 and then radiated for two minutes.
8. Eggs were treated as in 6 and then radiated for ten minutes.

The observations on these experiments made next day were as follows:

1. Membranes had formed, but there was no further result.
2. Some eggs had irregular membranes. There were no cases of division.
3. Disintegration had taken place. There were no membranes and no signs of division.
4. No evidences of division except a few small cells which looked like isolated blastomeres. One egg had a divided nucleus but showed no cytoplasmic constriction.
5. Disintegration had occurred. There were no membranes and no divisions.
6. Less disintegration was observed than in the preceding case. There were a few small cells.

7. Membranes were formed here; there were a few unsuccessful attempts at division, but none were complete.

8. This dish showed some membranes, some disintegration, but no signs of division.

Eggs from this same female when fertilized with sperm gave over 25 per cent normal swimming embyros, uniformly developed.

Thus ño artificial parthenogenesis was obtained with starfish eggs.

It is, of course, impossible to assert that artificial parthenogenesis cannot be brought about using X-radiation as a means until every one of the long series of agents already known with which the radiation might be tried shall have been used; and the present experiments make no claims of being an exhaustive attempt at this end. Rather the experiments here made were modifications of methods ordinarily successful. Yet it seems that if artificial parthenogenesis can be produced in this way, these experiments should have given more indication of it. When viewed in the light of conclusions from other experiments aimed at detecting permeability changes, the failure to produce parthenogenesis by this means seems significant.

On the basis, therefore, of the hypothesis current at this time that the initiation of division with which artificial parthenogenesis starts is due to changes in the permeability of the plasma membrane to cytolysing agents, the experiments to determine whether eggs can be induced to develop in this manner give evidence with their negative results for the conclusion that radiation does not cause any changes of importance in the egg's permeability.

In this connection a late communication by Loeb is of considerable interest. In accordance with his view that cytolytic agents produce artificial parthenogensis, Loeb tried the effect of ultra violet light upon Arbacia eggs. He succeeded in obtaining by this means in some cases fertilization membranes. Without further treatment, the eggs under-went cytolysis without segmentation; if the temperature was lowered, some divided once or twice before cytolysis occurred; "when the eggs were put for twenty minutes into hypertonic sea water, about ten minutes after the treatment with ultra violet light, they developed into

larvae," but scarcely went beyond the gastrula stage. Chaetopterus eggs exposed from five to ten minutes developed into swimming larvae without cell division. He expects Roentgen rays also to cause membrane formation since they too cause cytolysis. I have already noted cytolysis for starfish eggs.

The evidence from my experiments is hardly in agreement with Loeb's interpretation of his observations. It may be that the intensity of the rays which could be produced with my apparatus was not comparable with that of the ultra violet light, or that the two kinds of rays are not physiologically equivalent, but my experiments do not justify the hope that X-rays can be used as a means of producing artificial parthenogenesis. One cannot avoid raising the question, doubtless considered by Loeb himself, whether it was the ultra violet light which actually caused the parthenogenesis, in view of the fact that he has already shown that hypertonic sea water is enough to cause parthenogenetic development in starfish eggs, although he, to be sure, used sea urchin eggs for these experiments.

The indicator method. The indicator method for recognizing permeability changes has been made use of by various investigators but particularly by Harvey who has dicusssed it at length. The test is a more delicate one and the evidence given by it is of more value than that by either of the lines of investigation just discussed. The method involves the staining of the cells, starfish eggs in this case, with an intra vitam stain, which is sensitive to aklali, to act as an indicator, e.g., neutral red. When the concentration of the alkali is great enough or the permeability of the plasma membrane is increased so that the alkali may enter, its presence in the egg is indicated by a change of color from red to yellow.

It is true that when the alkali enters the egg it kills it and for this reason the method has been criticised; it has also been urged that the proteins in the cells as well as other substances present may influence the color change so that it becomes inaccurate and unreliable as a test of the rate of penetration of the dissolved substance into the cell. What the test really shows is the resistance of the cell to the entrance of the alkali. These criticisms

have been considered by Harvey ('13) and he reaches the conclusion as the result of renewed experiment that "the indicator method for the detection of alkali within the cell is therefore a perfectly adequate one." The alkali acts upon the surface of the cell changing the permeability before it enters and kills the cell body. And the difference in the rapidity with which the color change takes place for two alkalies indicates a difference in the permeability of the plasma membrane to the different alkalies.

The method for testing the present problem consists of finding the lowest concentration of the alkali (NaOH) which will just cause the color change. If radiation of the egg will cause increase in permeability, a less concentration of the alkali will then enter and the color change be brought about.

Starfish eggs which had been standing for some time (about 1 hour) were stained with neutral red. The eggs were divided into lots and three of them exposed to X-radiation for two, five and fifteen minutes. They were then tested with a solution of sodium hydrate in magnesium free sea water. (Of $\frac{1}{2}$ M. solutions of NaCl, KCl, and CaCl, 100 parts of NaCl, 2.2 of KCl and 2 of CaCl were taken for the sea water.) The sodium hydrate was diluted to the concentrations of $\frac{N}{100}$, $\frac{N}{200}$, $\frac{N}{400}$, and $\frac{N}{800}$, and the solutions were used to test first the control eggs (stained, but not radiated.) Between $\frac{N}{200}$ and $\frac{N}{400}$ was the concentration which caused the color change. To test the radiated eggs, it was necessary therefore to use only the concentration of $\frac{N}{400}$ and $\frac{N}{800}$. Immediately after radiation the same experiments were made on all of the lots radiated and no difference was detected in their reaction to the test. The same result was observed for all.

The $\frac{N}{400}$ solution did not cause any change in the control, a fresh lot of which was compared with every lot of radiated eggs, and neither this nor the $\frac{N}{800}$ caused a color change which was visible against white paper, but upon examining the experiment under the microscope the observer thought he detected a faint yellow "halo" appearing around the eggs with the first and perhaps to a less degree with both solutions. The reaction, however, was not of decisive character. Later the eggs faded and

died. Increase in permeability cannot be said to be positively indicated by this method, but if the results are to be interpreted as favoring its occurrence, certainly the increase is a slight one.

This test was repeated upon these same eggs about four hours later. No difference in reaction could be observed, indicating that if the radiation caused any increase it was not a permanent one.

The experiment when repeated upon the eggs which had stood four hours gave a similar result, although the yellow "halo" could now be seen about a very few of the control eggs when tested with the solutions of lesser concentration, suggesting perhaps, that the longer interval which elapsed before they were used had slightly lessened their resistance to the penetration of the NaOH.

The experiments and others which were like them on other days gave exactly similar results and established the fact that no marked change in the permeability of the eggs to NaOH followed the radiation, but it left open the question as to whether slight changes might not be caused which would account for the slight yellow "halo." In order to investigate this latter point a new series of experiments was performed exactly as before except that the stained eggs were mixed with a suspension of Chinese ink to bring out the jelly layer and to give a background against which to study refraction effects. The details of the experiment need not be given for the most painstaking observation showed no stain in the jelly and no change in the color of the radiated eggs where exactly the same changes could not be demonstrated in the controls. If the concentration of the NaOH solution was sufficient to penetrate the radiated eggs it was also sufficient to penetrate the control. This observation also led to the opinion that the "halo" was due to some other cause than radiation. No differences existed in the reactions of the two lots of eggs.

These observations all go to show that the radiation causes no change in the resistance of the membrane to the entrance of the alkali, and indicate no increase in permeability.

Elodea cells. The fourth method available for the investigation and the method upon which most confidence is placed

is a direct application of Harvey's experiments on Elodea leaves. This, it will be seen, is in principle the same as the test just preceeding. Elodea leaves which are mostly only two cells thick and, therefore, quite transparent, take up neutral red from a solution until they are quite red in appearance. The reasons for choosing Elodea are given in Harvey's paper. As he notes, the stain penetrates the cytoplasm and collects as a red solution in the sap vacuale, indicating a slight acidity of the sap. The cytoplasm and cell walls are usually left unstained. In this condition the leaves form excellent objects for the study of the penetration of various solutions. Many of Harvey's experiments the writer has verified; only that part which bears on the present problem is here discussed.

Harvey found that weak alkalies (NH_4OH and the amines) penetrate very rapidly while the strong (NaOH, KOH, etc.) experience resistance in entering. He further found that the entrance of the strong alkalies is facilitated by the addition of other substances (as chloroform, ether, urea, etc.) in small amounts. The completion of the reaction is shown by the "decolorization," the change from red to yellow of the leaves, and the reaction is a fairly sharp one. Red stained leaves were decolorized on the average in nineteen minutes in $\frac{N}{40}$ KOH + $\frac{M}{8}$ urea in distilled water. If, now, radiation caused change in the permeability of this cell there should be a difference in the times of decolorization of the radiated and non-radiated leaves.

Of all the alkalies tried, a solution of $\frac{N}{40}$ KOH + $\frac{M}{8}$ urea gave the most clearly cut reaction. Red stained leaves were placed in this solution immediately after being radiated for varying lengths of time of from two to fifteen minutes duration. Whole leaves were tested; they were taken from the same or adjoining whorls, for Harvey has shown that widely separated leaves or those from different branches are not comparable. In no case could there be distinguished any difference in the time required for the decolorization of the non-radiated control leaves, the short and the long radiated ones. The average time required, according to my experiments (and this is in agreement with Harvey's data) is nineteen minutes. In a variation of this

experiment the red stained leaves were placed under the X-ray tube while in the alkali solution. Even the constant radiation during the action of the alkali on the leaves did not cause any difference in the time necessary for penetration as indicated by the decolorization of the leaves. Repeated trials of these experiments have given exactly the same results. The rate and penetration of this solution into the Elodea leaves is not changed under the influence of radiation. The conditions are the same where the alkali acts without the added substance. Only a longer time is required for the penetration.

Other of the stronger alkalies act in the same way. It is impossible to distinguish any difference in the rate of penetration into radiated and non-radiated leaves.

Only thirty seconds are required for the penetration of $\frac{N}{40}$ NH_4OH into Elodea cells. Radiated leaves also are decolorized in the same time.

The entire series of experiments on the relative rates of penetration of various alkalies into red stained Elodea cells gave uniformly the same result: the radiation whether shorter or longer does not influence the rate; or, in other words, the radiation brings about no changes in the permeability of the cells for the alkalies under investigation.

Conclusion and discussion. The experiments which have been set forth all warrant the conclusion that the effects which are described by numerous workers as the result of exposure to X-rays are not to be attributed to permeability changes caused by the radiation. Arenicola embryos do not exude pigment under the influence of the radiation. It has not been found possible to induce artificial parthenogenesis with X-rays used either as a first or a second treatment; according to current interpretations artificial parthenogenesis implies changes in permeability. Starfish eggs stained in neutral red are decolorized equally rapidly by sodium hydrate whether or not they have been radiated; and the same thing is true of Elodea leaves and KOH. Evidence from all these lines is in perfect agreement as indicating that no permeability changes are caused by the radiation. The experiments from which the evidence is drawn included only a limited number of substances, it is true, and no

sweeping generalizations may be made from them; but the conclusion is clear that for the substances used no change in the permeability of the plasma membranes is brought about by X-radiation. To assume such changes for other substances is not warranted by the evidence at hand.

Gager's experiments perhaps bear on this problem somewhat. He reports that he obtained only negative results with regard to the effect of radium rays on osmosis, turgidity, and consequent cell enlargement.

While there are certain analogies as mentioned earlier for the causation of permeability changes by radiation, there are at least two facts of common clinical experience which would not lead to that expectation. First, protoplasm is transparent to X-rays; the surface of the cell does not present an obstacle to the passage of the rays, which on the contrary are able to act some distance from the surface of a tissue mass. This, of course, does not preclude the possibility of changes in permeability being induced, but it indicates that the changes are not necessary to the action of the rays. Second, the rays are able to act on cells and tissues which are not bathed by solution. For example, extensive injuries on the skin are caused by exposure to the rays and they clearly do not depend on the entrance of anything to which plasma membranes are permeable into the cells. Neither of these facts precludes the possibility of permeability changes, but both are consistent with the idea that such changes do not occur following radiation.

The trend of physiological investigations in recent years has been away from the idea that particular agencies of experiment cause specific effects on organisms; that is, the same experimental result can often be gotten by various means, as is illustrated by the work on artificial parthenogenesis. On the other hand, some, at least, of the effects of radiation seem of a different character from those of other agencies. Now many of the various means used for causing departures from the normal cycle of events in organisms are known to act by causing permeability changes. It seems not illogical to suggest that the apparent specificity of radiation, in so far as it is real, may be due to the fact that it does not cause such changes, and is not able there-

fore to set up the same series of processes of cytolysis and the like as takes place following action by the more familiar agencies.

Since experimental investigation does not warrant the conclusion that radiation acts by causing changes in the permeability of cells, we must look to other causes for explanation of its effects. The Hertwigs and others have shown that chromatin is injured by the rays and it has also been proven possible to effect the activity of enzymes by radiation. Packard has even suggested that the chromatin injuries are due indirectly to the effect on cell enzymes, and Miss Woodward and the writer have established the capacity of X-rays to modify the activity of the egg extractive, fertilizin. The evidence available at the present time points to a theory of enzyme modification as the best explanation of the effects of radioactivity upon the structure and functions of protoplasm.

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THE VASOTONIC AND THE VASOREFLEX CENTRE

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I

In 1910 the writer questioned the belief that both the arterial tonus and the vasomotor reflexes are controlled by the same master cells, the so-called vasomotor centre. He pointed out that this conception was purely an hypothesis—a far-reaching hypothesis, for if the vasotonic and the vasoreflex centre are identical, the measurement of the vasomotor reflexes will reveal the condition of the apparatus for the maintenance of both functions, the tonus as well as the reflexes.

In that investigation[1] two methods were used to test the truth of the hypothesis. These methods indicated that the tonus and the reflexes were not controlled by the same nerve centre. Indeed they seemed to indicate that the bulbar cells did not modify the vasomotor reflexes. Yet the investigator expressly declined to go further than the conclusion that his work marked the speculative character of the hypothesis under examination and showed the need of further research to justify its acceptance.

This caution was well grounded. The problem in hand must at present be classed with those upon which a working decision should be made but which apparently cannot be decided by a crucial experiment. In such cases we must weigh the evidence pro and con and take the more probable side. The degree of probability depends naturally on the character of the observations and on the number of experimental methods that can be made to converge on the point attacked. In the previous

[1] W. T. Porter. This journal, 1910, xxvii, p. 276.

investigation but two methods were employed and their combined evidence was far from being conclusive. It was for these reasons that the writer reserved judgment.

The present communication brings forward a third method, the results from which agree with those from the first and second methods in that they apparently separate a vasotonic and a vasoreflex centre but differ in that the third method shows the reflexes actively affected by the condition of the vasoreflex centre.

II

The method now offered consists in applying a single reagent to the alleged single vasomotor centre. If the centre be indeed single, the changes produced by the reagent in the two functions of the centre should be in the same direction. When the reagent increases the tonus it should also increase the reflex. If both reflex and tonus are the results of the energy of one and the same nerve cell, both functions should be augmented or depressed as the energy of the cell is augmented or depressed. But curare, the reagent employed in these experiments, does not produce this reaction. It can be so administered that one of these functions is altered while the other is unaltered or is altered in the opposite direction. Curare thus separates the vasoreflex from the vasotonic function.

III

The experiments were performed on cats and rabbits. Following are typical protocols, selected from sixteen successful animals.

Experiment November 6, 1914. I. In an etherized cat the carotid blood pressure was recorded with a membrane manometer, the graduation scale of which is shown in figure 1. At 2.10 p.m. curare was given by the external jugular vein and the central end of the divided sciatic nerve was stimulated at intervals of five minutes. The secondary current was distinct when the electrodes were placed on the tongue. The sciatic reflex rose from 30 to 60 mm. while the tonus scarcely varied.

II. At 3.30 p.m. the cat had excreted the greater part of the curare and the experiment was repeated. Curare was again injected and the sciatic nerve was stimulated at ten-minute intervals. The result is

Fig. 1. About nine-tenths the original size. Carotid pressure in the cat, November 6, 1914, after the injection of curare. The sciatic nerve was stimulated at intervals of ten minutes. The reflex rise increases greatly, while the tonus remains almost unchanged.

Fig. 2. About four-fifths the original size. The carotid pressure in the rabbit after the injection of curare. Reading from left to right, the depressor nerve was stimulated at 3, 3.05, 3.15 and 3.25 p.m., November 20, 1914, and in another rabbit at 10.31, 10.51 and 11.11 a.m., November 19. The depressor reflex greatly increases, while the tonus is little changed.

shown in figure 1. The curare caused the tonus to fall from 108 to 90 mm., at which level it remained almost constant; the reflex diminished from 42 to 30 mm. and then increased to 67 mm.[2] The individual measurements in this and the two following experiments are shown in table 1.

TABLE I

The carotid blood pressure before and during stimulation of the sciatic and the depressor nerve at successive intervals of 5 or 10 minutes following the injection of curare

CAT. SCIATIC REFLEX				RABBIT. DEPRESSOR REFLEX			
Date	Before stimulation	During stimulation	Absolute rise	Date	Before stimulation	During stimulation	Absolute fall
November 6.....	110	140	30	November 19.....	90	58	32
I	110	142	32		93	39	54
	113	150	37		100	40	60
	110	151	41		108	38	60
	110	155	45		100	32	68
	111	154	43		90	32	58
	110	158	48		99	36	63
	110	150	40				
	106	148	42	November 20.....	108	90	18
	108	160	52		88	57	31
	100	160	60		90	48	42
	106	162	56		90	38	52
November 6.....	108	150	42				
II	90	120	30				
	92	138	46				
	90	142	52				
	85	141	56				
	90	143	53				
	95	141	46				
	90	157	67				

Experiment November 19, 1914. The carotid pressure of a rabbit was recorded. At 10.25 a.m. the depressor nerve was stimulated. At 10.26 curare was injected. At 10.31 and every ten minutes thereafter until 11.31 a.m., the depressor nerve was again stimulated. On the

[2] In order to shorten figure 1, the kymograph was stopped an instant after the reflex rise began and was released an instant before the maximum rise was obtained.

right hand side of figure 2 are shown the reflexes obtained at 10.31, 10.51, and 11.11 a.m.

In this experiment, the tonus remained almost constant, while the depressor reflex increased from 32 to 68 mm.

Experiment November 20, 1914. A rabbit was curarized at 2.55 p.m. and the fall in blood pressure on stimulation of the depressor nerve was measured at 3.00, 3.05, 3.15, and 3.25 p.m. The carotid pressures before stimulation were 108, 88, 90, and 90 mm. In other words, the tonus fell slightly and then remained practically constant. The depressor reflex, as shown at the left of figure 2, increased from 18 to 52 mm.

IV

These experiments show that curare may more than double the sciatic and the depressor reflex change in blood pressure while the arterial tonus is left substantially unchanged. It seems impossible to reconcile these results with the present conception of the vasomotor centre. Unless this can be done, it will be necessary to accept a vasotonic and a vasoreflex centre, related but separable.

THE EFFECT OF PARTIAL ADRENAL DEFICIENCY UPON SYMPATHETIC IRRITABILITY

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In 1904 Elliott (1) reported the observation that in a cat moribund after complete adrenal extirpation the pressor reaction to nicotin was abolished and concluded that under such circumstances the irritability of the sympathetic system is lost. He offered the suggestion that adrenal deficiency results in a corresponding deficiency in circulating epinephrin which in turn renders the sympathetic myoneual junctions incapable of transmitting impulses. In 1909 Gautrelet and Thomas (2) reported results obtained in a dog and a rabbit that supported Elliott's conclusion. Last year Hoskins and Wheelon (3) further investigated the matter. They thought that animals near the point of death are scarcely capable of giving significant information. Accordingly they studied the condition of the vasomotor mechanism in dogs during the earlier hours after ligation of both adrenals. They hoped thereby to detect the primary effect of adrenal deficiency before secondary results had obscured the picture. In such experiments they were unable to find any evidence of sympathetic depression at a time when the animals were showing marked muscular and cardial weakness. Their method of attacking the problem was based upon a supposition that the organism is not significantly affected by the loss of any quantity of adrenal tissue short of that which causes death. That idea receives a certain amount of support from such experiments as those recently reported by Crowe and Wislocki (4). These investigators found that glycosuria is caused by the manipulation of any fragment of adrenal tissue that is sufficient to maintain life,

but that equal irritation in the adrenal region in the absence of the gland tissue is without effect.

Whipple and Christman (5), however, have recently shown that partial adrenal deficiency causes a decrease in the amount of phenoltetrachlorphthalein excreted into the intestine, a result which they attribute to hepatic depression.

In the light of these results it seemed desirable to study the effect of partial adrenal deficiency upon vasomotor irritability. In order to obtain results as well marked as possible an attempt was made in the earlier experiments to reduce the adrenal tissue to the lowest amount compatible with survival. In a series of seventeen dogs one adrenal—usually the right—was destroyed completely, and at the same operation one-half to three-fifths of the other gland was similarly treated. An epidemic of distemper, added to the severity of the operation, gave a high mortality in the series but five of the animals survived. This series was supplemented by six successful cases in which the left adrenal only was destroyed.

The general methods employed in the research were the same as those previously described by Hoskins and Wheelon (6). Blood pressure from a femoral or carotid artery was recorded by means of a mercury manometer, using a reservoir cannula filled with 10 per cent sodium citrate. The reaction to a standard dose of adrenalin gave an index of the condition of the peripheral vascular structures. Similarly the reaction to nicotin indicated the degree of irritability of the sympathetic system proper. After the reactions to adrenalin and nicotin were obtained the vessels were ligated and the incision closed and dressed with a piece of gauze saturated with flexible collodion. In view of the fact that the whole procedure of setting the cannulas and closing the wound requires less than 10 minutes it did not seem worth while to attempt to surmount the difficulties of a "bloodless" technique. Aseptic precautions were taken throughout. Various methods of destroying the adrenal tissue were tried: excision, actual cautery, interstitial injections of chloroform or chromic acid and simple ligation. The injection methods were unsatisfactory in that they were hard to control. The liability to hemorrhage

and danger of injury to splanchnic nerve trunks rendered the cautery unsatisfactory. Excision of the adrenals without undue injury to nearby structures is notably difficult and time consuming. Ligation, on the other hand, is easily accomplished and is equally effective. This statement is based upon two observed facts: the reaction of the animal to ligation is characteristic of adrenal destruction and subsequent examination of the ligated glands shows that they have undergone destructive degeneration. In both these respects the present research has confirmed the observations of earlier investigators (7). The technique employed in isolating the glands was the following: The peritoneum over the adrenal was torn loose and by blunt dissection the organ was partially loosened from contiguous structures. Particularly it was sufficiently separated from the sympathetic trunks that subsequent tightening of the ligatures would not injure these. Two strands of strong linen thread were then passed together longitudinally under the gland. These were brought up, one on either side, and tied. The gland was thus completely isolated. This procedure is especially advantageous in case of the right adrenal which in the dog usually lies well under the vena cava. A bit of the dorso-lateral wall of the cava was grasped in a hemostat. Lateral traction then rolled the vein off the gland and left it fairly accessible, while offering no serious impediment to the circulation.

Parenthetically, it may be permissible to allude again to the advantages of the reservoir cannula method in routine blood pressure work. The cannula we have finally come to use with dogs is one of the ordinary arterial type in which is blown a bulb holding about 15 cc. This is filled with 10 per cent sodium citrate solution and attached directly to the manometer. The method avoids the inconvenience of developing an initial positive pressure in the system and of maintaining a cumbersome "wash-out" arrangement. When the artery clip is released an outflow of blood forces the citrate from the central stem of the cannula and partially displaces that in the bulb until pressure equilibrium is reached. The animal is thereby protected from a not uncommon accident—an intravascular injection of the anti-coagulant

when the artery is opened. Before coagulation has had time to occur, the citrate makes its way back against the blood column in adequate quantity to prevent clotting, but not enough reaches the circulation to cause any perceptible effect. This is true even though a considerable fall of blood pressure occur. It is rarely necessary to remove the cannula and dispose of a clot even in experiments lasting two hours or longer. The simplicity of the method renders it particularly advantageous for students' use in conventional blood pressure experiments. The only precaution we have found necessary is to avoid the use of too large a reservoir.

At intervals of one to eight days in various cases, after adrenal operation the blood pressure and reactions to adrenalin and nicotin were again determined. The results indicate that partial adrenal deficiency does result in sympathetic depression. Experiment No. 4 which illustrates the general outcome of the series will be described:

November 24, 1914. Dog, Female adult. Weight, 7 kilos. Cannulas set in right femoral artery and vein. Reactions determined to adrenalin 2 cc. 1–200000, nicotin 0.8 cc., 1:4000, pilocarpin 0.5 cc. 1–10000.

Laparotomy. Abdomen opened in median line. Right adrenal gland exposed and ligatures placed so as to isolate posterior half of gland—circulation of anterior half apparently not harmed. Right gland exposed and completely isolated with two ligatures. Incision closed in three layers. Excellent recovery.

November 27. Dog somewhat weak as judged by resistance to anesthetizing. Incisions in leg and belly wall clean. Cannulas set in left femoral artery and vein. Reaction to adrenalin, nicotin and pilocarpin obtained as before.

December 3. Dog weak. Incisions not well healed but apparently not purulent. Cannulas in carotid artery and external jugular vein. Reactions to adrenalin, nicotin and pilocarpin taken as before. Animal killed.

Post mortem findings: Right adrenal: Anterior half of gland apparently normal: Posterior half degenerated, largely replaced by sclerotic tissue. Left adrenal: Marked central liquifaction necrosis leaving thin superficial layer of soft brownish yellow tissue.

Right and left splanchnic nerve trunks traced through operative fields. No evidence of their having been injured in the operation.

Subsequent measurement of the tracings in this experiment showed that the original blood pressure was 146 mm. Three days later at the time of the second determination it was 132. Nine days after the adrenal ligation it was still lower, 110 mm. The nicotin reactions were respectively 50, 22 and 14 mm. The pressor reaction to adrenalin was unusually constant being exactly 40 mm. in each case.

In several instances the blood pressure reactions to small doses of pilocarpin were determined, before and after adrenal ligation. No significant differences were observed. Apparently, therefore, the lessened irritability of the sympathetic system is not shared by the para-sympathetics.

A possible source of error in such experiments is the nearness of the splanchnic nerve trunks to the adrenal glands. Injury to these nerves might well cause perturbations in the vasomotor reactions. Elliott has shown, however, that decentralization of sympathetic paths results in heightened irritability to adrenalin. If, therefore, injury to the splanchnic trunks were a significant factor in our results depression of the nicotin reaction should be accompanied by augmentation of the adrenalin reaction—a condition that ordinarily did not maintain.

No attempt was made to determine exactly the minimal quantity of adrenal tissue that must be removed to cause an appreciable loss of sympathetic irritability. Owing to individual variability in this respect a large series of experiments would probably be required to settle the point. In some cases we noted, however, that removal of one gland only was without effect, while in others a depression resulted. It is probable, therefore, that the "margin of safety" is about 50 per cent.

Considering that the vasomotor depression resulting from adrenal deficiency might conceivably be due to a reduction in the amount of circulating epinephrin the effect of slowly supplying adrenalin to the blood stream seemed worthy of investigation. Accordingly in two animals that showed well marked depression

in the reaction to nicotin dilute adrenalin was infused for half an hour into a vein. The results were surprising. Even though the infused adrenalin was producing little or no effect upon blood pressure the reaction to nicotine soon became smaller and when the rate of infusion was increased to cause a minimal pressor effect the nicotin reaction was abolished. These results tend to indicate that epinephrin deficiency is not the cause of the sympathetic depression resulting from adrenal deficiency. The phenomenon is being further investigated and results will be reported in a later communication.

For the apparent discrepancy between the previous results of Hoskins and Wheelon and those herein reported no definite explanation is offered. It would seem, however, that in their experiments the overwhelming severity of a laparotomy added to the immediate effects of total deprivation of adrenal tissue caused a primary failure of the cardiac metabolism before the sympathetic system had time to be significantly affected. Also the possibility exists that the depression of sympathetic irritability observed in the experiments herein reported is not at all specific, but merely one phase of general depression of vitality, such as occurs in Addison's disease. The sum total of available evidence seems to indicate that the essential feature of adrenal deficiency is an interference with fundamental metabolism—possibly oxidation—in which the more active tissues of the body suffer first.

SUMMARY AND CONCLUSION

From one-half to seven-tenths of the adrenal tissue was removed from dogs in various cases, at a single operation. At intervals of one to eight days after the operation the blood pressure and the vasomotor reaction to nicotin were decreased. The reaction to adrenalin was not similarly affected. Partial adrenal deficiency therefore results in a depression of the irritability of the sympathetic nervous system proper. This depression is probably only one phase of a generalized interference with fundamental metabolism.

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(7) MOSSOU ET LE PLAY: Comptes rendus de la société de biologie, 1909, p. 36, p. 83; ALLEN: Glycosuria and Diabetes, Boston, 1913, p. 863; HOSKINS AND WHEELOU, loc. cit. (3).

THE INVERSION OF RESPIRATORY WAVES IN SPHYGMOMANOMETER RECORDS OF ARTERIAL PRESSURE IN MAN

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In their work on the indirect determination of blood pressure Howell and Brush[1] showed experimentally among other things, that maximum oscillations of the sphygmomanometer lever obtain when the extra-arterial pressure of the instrument equals the intra-arterial pressure during diastole.

Since then as is well known various instruments have been devised for the express purpose of bed-side determination of diastolic as well as systolic pressure. One of the earliest of these instruments, and one which still proves to be most satisfactory in this laboratory, is the Erlanger sphygmomanometer.[2]

Those who are familiar with these instruments and with the graphic records obtained from them are also familiar with the respiratory waves which appear almost constantly in them.

During routine instruction the writer's argument for the interpretation of these respiratory waves has been somewhat as follows:

1. Observing a continuous graphic record of the sphygmomanometer with external pressure gradually falling from a point somewhat above systolic to a point somewhat below diastolic, one notes that the excursions of the writing point at first increase from smaller to greater height, then having reached a maximum fall again to smaller dimensions.

2. The region of greatest excursions in general indicates an equilibrium between the external pressure upon the artery and the internal pressure during diastole. The more the external pressure deviates

[1] Howell and Brush: Proceedings of the Massachusetts Medical Society, 1901, xviii, pp. 655–672.

[2] Erlanger, J.: This journal, 1902, xii, p. 53.

from the internal (diastolic) pressure the smaller become the excursions; the more nearly the external pressure approaches internal (diastolic) pressure the greater become the excursions (Howell and Brush, 1901).

3. With the external pressure kept constant at any given point the excursions of the sphygmomanometer lever ought also to remain constant provided no change of the internal pressure of the artery occurs. Such a condition of internal pressure however rarely obtains. The so-called respiratory waves in the graphic records are familiar evidences of this fact.

4. With the sphygmomanometer pressure set at a point near mean blood pressure the respiratory waves, as usually observed, are most pronounced. The part of this wave made up of increasing excursions must be indicative of rising internal pressure. For since the external pressure remains constant and considerably above the lowest diastolic, and since increasing excursions indicate approach toward equilibrium of the two pressures, the lower, but variable, internal pressure must be increasing toward the higher, but fixed, external pressure.

Conversely the part of the respiratory wave made up of decreasing excursions must be indicative of a falling internal pressure.

5. If together with the sphygmomanometer record the respirations of the subject are graphically recorded, then it is possible to determine what part of the respiratory wave in the blood pressure trace corresponds to the inspiratory act, what part to the expiratory act (Erlanger, 1905).[3]

With such a record one may demonstrate whether there is inspiratory rise and expiratory fall of blood pressure in the subject, or *vice versa*; or whether the changes are of a more complicated character. Indeed Erlanger and Festerling[4] have used this method in a study of the effect of respiratory movements upon blood pressure in man.

6. As a logical consequence of the above argument the following proposition ought to hold good, and ought to be borne out by experiment:

If the sphygmomanometer pressure on the arm be set at some point *below* diastolic pressure, instead of *above* as is usually done, one ought also to obtain respiratory waves in the record. *Only the waves then would appear inverted when compared with those in the record taken with external pressure above diastolic.*

[3] Erlanger: Journal of Experimental Medicine, 1905, vii, p. 713.

[4] Erlanger and Festerling: Journal of Experimental Medicine, 1912, xv, p. 370.

One ought to be able to demonstrate this inversion of respiratory waves by simply recording the respiratory movements graphically and simultaneously with the sphygmomanometer record. Care need only be taken to have the two writing points in the same vertical line.

Having determined the diastolic pressure of the subject in the usual way, one record should be taken with the external arterial pressure set above the diastolic, another record taken with the external arterial pressure set below the diastolic pressure.

In preliminary tests with students it was very gratifying to find that this inverted respiratory wave could actually be obtained, and the test was incorporated as one of the accessory exercises of the laboratory.

As will be seen below most of the subjects, so far carefully examined, show the inverted wave in their sphygmomanometer records.

The chief significance of this inverted respiratory wave, at this point of our consideration, is two-fold. (1) It demonstrates deductively the validity of the general proposition that maximum excursion of the sphygmomanometer lever (Erlanger instrument) is indicative of diastolic pressure. (2) It furnishes a method for the determination, and interpretation, of blood pressure changes in the respiratory waves of the sphygmomanometer record.

Before speaking of the experimental results the writer wishes to state, in the interests of priority, that while the idea of the inverted respiratory wave and its demonstration had been independent on his part yet he had been anticipated by Erlanger and Festerling.[5]

In the early part of the observations taken in this study the writer in conversation with one of these authors (Erlanger) discovered that they too had seen the logical consequences of Erlanger's earlier work (1905).

However, Erlanger and Festerling only observed the inverted wave upon the "arteriograph" records taken on the exposed artery of a dog. In man they were wholly unable to observe it.[6]

[5] Erlanger and Festerling: Loc. cit., pp. 380-381.

[6] Ibid., p. 384. "In the case of man it seems that the inversion of the waves of oscillation associated with respiration does not take place. . . . ."

The results here reported are therefore new in the sense that they show the presence of an inverted respiratory wave in the sphygmomanometer trace of man.

EXPERIMENTS

Method. The devices employed hardly need description beyond the statement that care was taken to avoid leaks in the closed air spaces of the transmitting tambours, tubing, etc., and to have the tambours themselves covered with rubber dam of the proper resilience.

As stated above the Erlanger sphygmomanometer was used. Whenever a larger recording surface was desired, that of an ordinary kymographion drum was substituted for the small drum supplied with the sphygmomanometer.

In the latter experiments the recording tambour transmitting the respiratory movements was supplied with a lever writing in a vertical line. Ludwig's form with jointed writing tip (used with the lever arm directed at right angles to the tangent-plane of the drum) was employed in some of the experiments. Such a writing tip, it will be remembered, traces the *chord* of the arc described by movments of the lever arm.

In other experiments a writing lever specially devised by the author was employed. This lever consists of a short writing tip suspended from the end of a thread which in turn is held on the grooved rim of a light wheel of 12 cm. radius. To prevent lateral oscillations of the writing tip it is suspended within guide posts. A smaller wheel of 12 mm. radius is rigidly attached to the axis of the larger wheel; a thread suspended from the rim of the smaller wheel is fixed to the recording tambour. The wheel is set up so that its movements are through the vertical plane. The writing tip thus records the *tangent* of the arc described by the movements transmitted from the tambour. In contradistinction to the Ludwig *chord describing* lever this instrument may be known as a *tangent recording* lever, and will be so referred to in this paper.

The advantages of a lever recording vertical lines rather than arcs are too obvious to merit further mention.

In all records where synchronous points of two or more tracings are to be determined "scratch marks" are indispensable. Accordingly no records in the present study were considered that do not bear such scratch marks.

The receiving tambour for the respiratory movements was either a pneumograph devised by Howell consisting of a rubber-bag (made of a section of inner bicycle tubing) applied directly to the chest walls. A modified Marey's sphygmoscope is inserted in the path of transmission of this apparatus. At times the pneumograph of P. Bert (simple metal cylinder with rubber dam heads), or that of Marey (steel spring plate) was used. Care was taken to apply the pneumograph to the chest-walls and upper abdomen in such manner as to enable the instrument to record promptly and faithfully the respiratory movements.

It should here be stated that in order to make the inverted respiratory wave stand out prominently upon the record the rate of respiration must be slow enough to include 6–10 heart beats, or even more. Slow, deep breathing therefore is the rule. It may be also added that the inspiratory phase should be of a duration equal to that of the expiratory phase, and if possible pauses between the two phases should be avoided.

Results. The observations upon which this study is based were made partly in the spring of 1913, partly in the fall of 1914. Many observations were made by students of the present third and second years, medical department, under the writer's supervision.

The men were of normal health and of ages varying for the most part between twenty-two and twenty-eight years.

The writer wishes to take this opportunity to thank these gentlemen who thus have assisted in the collection of material for this work. Especially does the writer wish to thank Messrs. Rice, Shipton and Stifel of the second year class for the technically perfect and beautiful records which they have kindly donated to the study.

Up to the present writing twenty-eight persons in all have been satisfactorily examined for the inverted respiratory wave. Of this number twenty persons showed an inversion of the

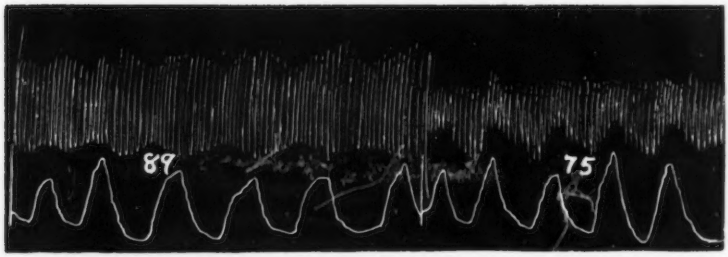


Fig. 1. Subject R. B. S. Diastolic pressure, 82 mm.; systolic pressure, 120 mm.; tangent recording respiratory lever, upstroke indicates inspiration; the numbers in the figure, 89 and 75, indicate the external pressure applied to the brachial artery during the experiment.

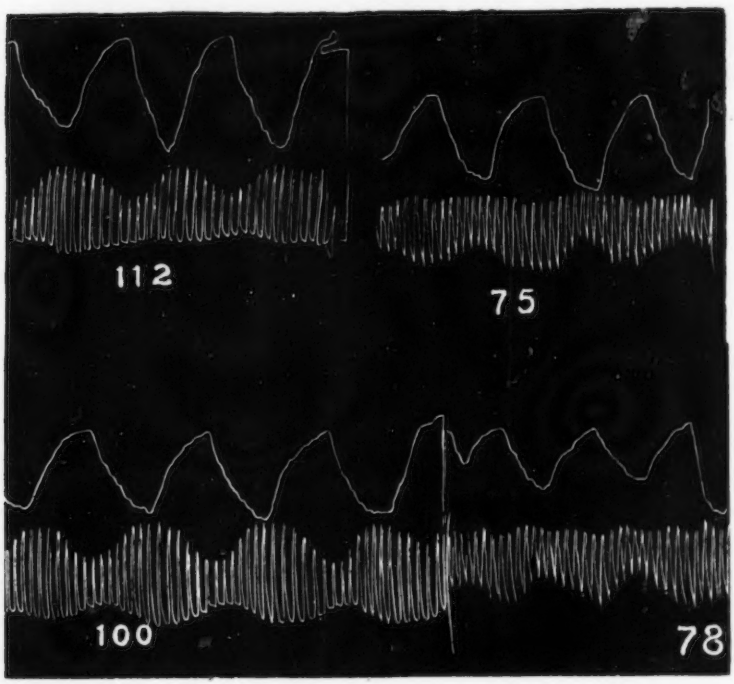


Fig. 2. Subject C. D. S. Downstroke of the respiration lever (Ludwig's lever) indicates inspiration. The numbers below each section of the tracings indicate the extra-arterial pressures applied at each reading, the pressures 100 and 112 being above, the pressures 78 and 75 being below diastolic pressure (88 mm.)

respiratory wave, and eight failed to show it. It was further observed that some individuals who showed the inverted wave at one time, at another time failed to show it. It is possible, therefore, that if the eight individuals who failed to show the wave had been examined under different conditions, they too would have shown the inverted respiratory wave.

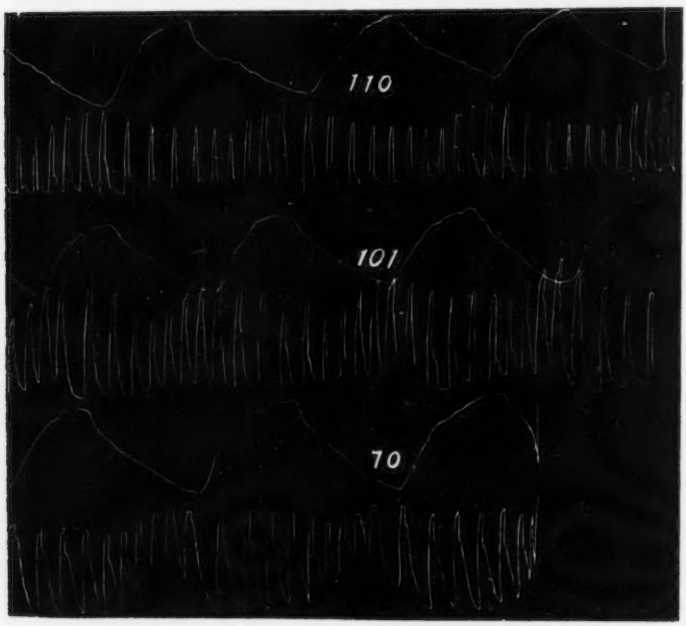


Fig. 3. Subject E. H. C. Diastolic pressure, 96 mm.; downstroke of respiration lever indicates inspiration. The numbers indicate the external pressure in the cuff of the sphygmomanometer at each of the three readings.

Indeed an analysis of the records in regard to change of heart rate and blood pressure within the respiratory cycle seems to indicate that the failure to demonstrate the inversion of the respiratory wave may be due to a certain physiological complex prevailing only at the time.

For example, of the twenty cases showing inversion of the respiratory wave eighteen could be classed as having rise of blood-pressure chiefly during inspiration, fall of blood-pressure during expiration. Of the eight cases failing to show inversion of the respiratory wave six could be classed as having fall of blood pressure chiefly during inspiration, rise of blood-pressure chiefly during expiration. From the premises stated above one ought to obtain the inverted respiratory wave regardless of the type of blood-pressure change associated with the respiration. The point evidently requires further investigation.

Specimen records showing the inversion of the respiratory wave in the sphygmomanometer are here reproduced. The inversion is seen readily if one compares sections of a record, one of which was made with the external arterial pressure above, another of which was made with the external arterial pressure below diastolic.

In figure 1, record of R. B. S., comparing the two sections shows the inversion. The one section was made with external pressures at 89, the other section was made with external pressure at 75 mm. The diastolic pressure of this individual at the time was 82 mm. Hg.

In figure 2, one compares the sections with external pressures set 112 and 75, or the sections with external pressures of 100 and 78 mm. The diastolic pressure of this individual was about 88 mm.

Again in figure 3, one compares the middle (or the upper) with the lower section. The diastolic pressure of this subject at the time was 96 mm. The external pressure on the arm in the upper section was 110, in the middle record 101 and in the lower record 70 mm.

One now will ask how much above and how much below the diastolic pressure must the pressure in the cuff be set to ensure an inversion of the respiratory wave. The answer to this would be, theoretically, not more above nor below than the amount of blood pressure change accompanying the respiration. Practically one sees in figure 1, that the two pressures between which inversion took place are pretty close together, just 7 mm. above

and 7 mm. below the diastolic pressure as determined at the time.

If one take a record with the escapement set so as to allow the pressure in the cuff to fall gradually one ought to find in the record the exact point where the inversion takes place. This has been done and is shown in figure 4. The upper sections of the record show records with external pressure constant at three different levels. Inspection will show the respiratory wave to have undergone inversion. The lower and more important part of the figure is a record of the same individual taken with external pressure gradually falling.

As will be noted the point where the inversion occurs is between 90 and 95 mm. pressure in the sphygmomanometer cuff. The inversion here, as in most cases studied, takes place at the region of last maximal oscillations.

In this study, at any rate, the last maximal oscillations has always been the criterion of diastolic pressure, regardless of sounds.

A further point is suggested by the record shown in figure 4. If the inversion of the respiratory wave in the blood-pressure trace takes place at diastolic pressure may it not be that this point of inversion itself at times could be profitably used as an additional test as to the correct diastolic pressure in man? In figure 4, at 95 mm. no inversion has yet taken place while at a point just below 90, say 88 mm., the inversion is already accomplished. Clearly in this case 90 mm. is very near the exact point of inversion. The region between 90 and 95 is the region of last maximal oscillation and therefore it is clear that the region of inversions and the region of true diastolic pressure may be one and the same in terms of pressure.

SUMMARY

1. An argument is submitted demonstrating deductively the following proposition:

In case the actual respiratory movements in man are synchronously and graphically recorded together with the sphygmomanometer trace, and with the extra-arterial pressure set at

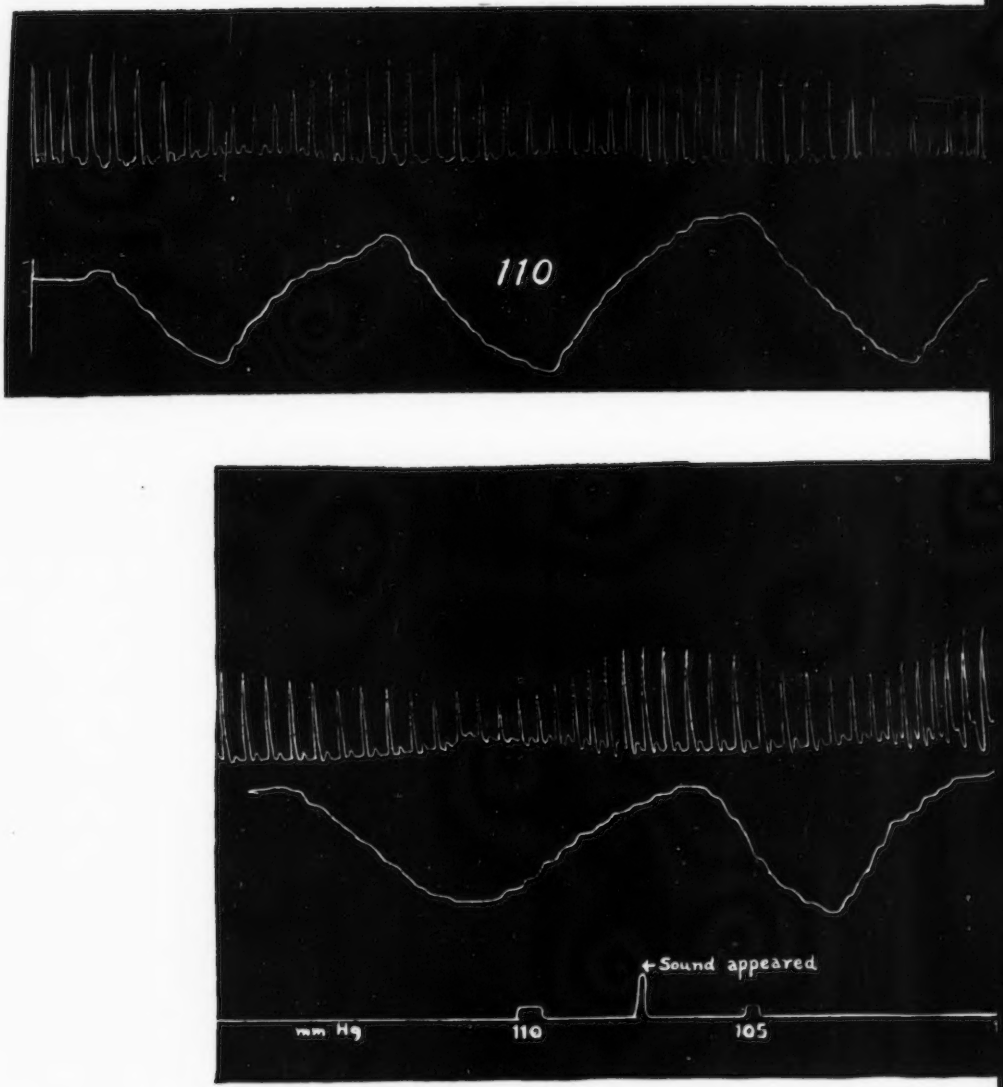
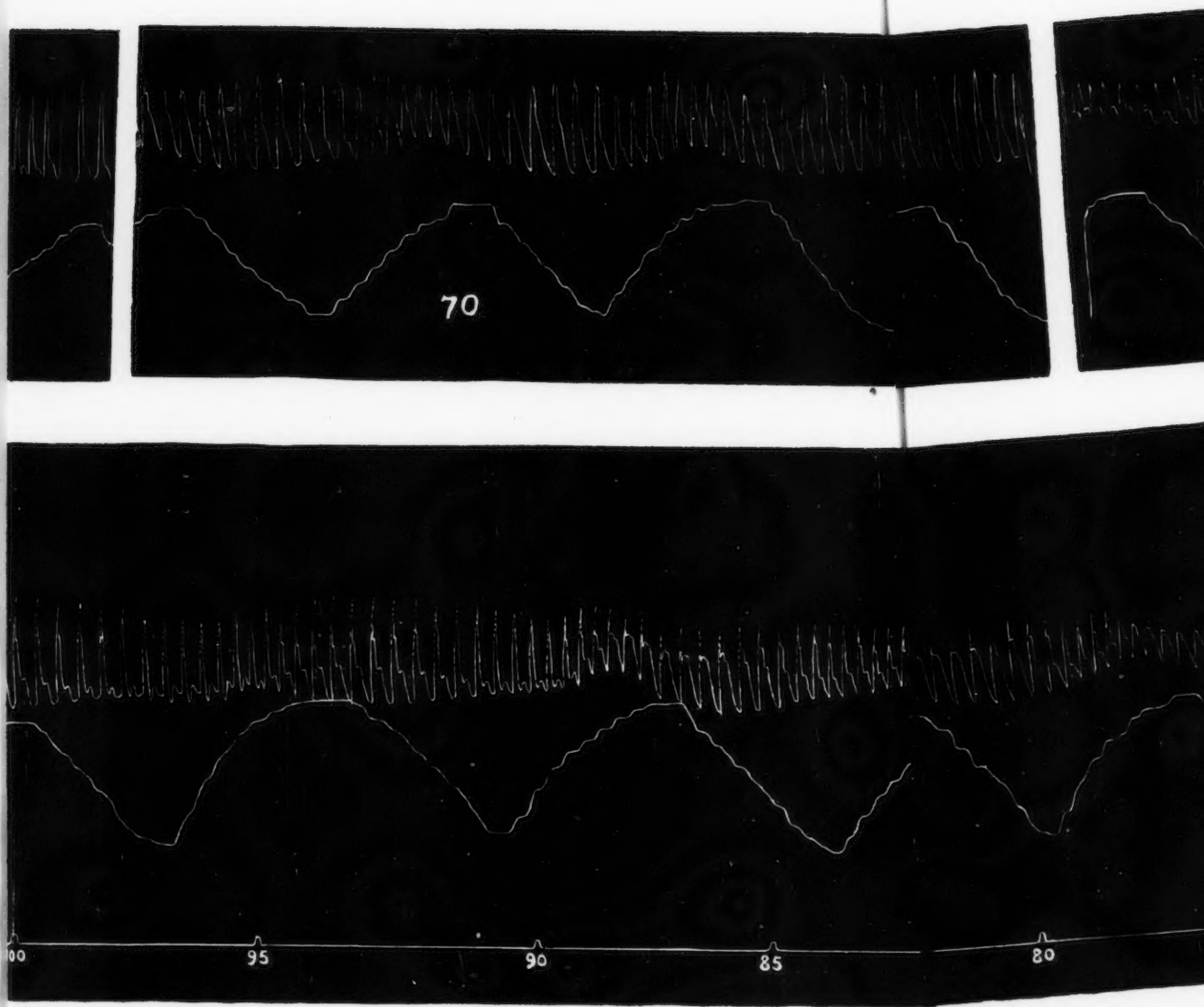
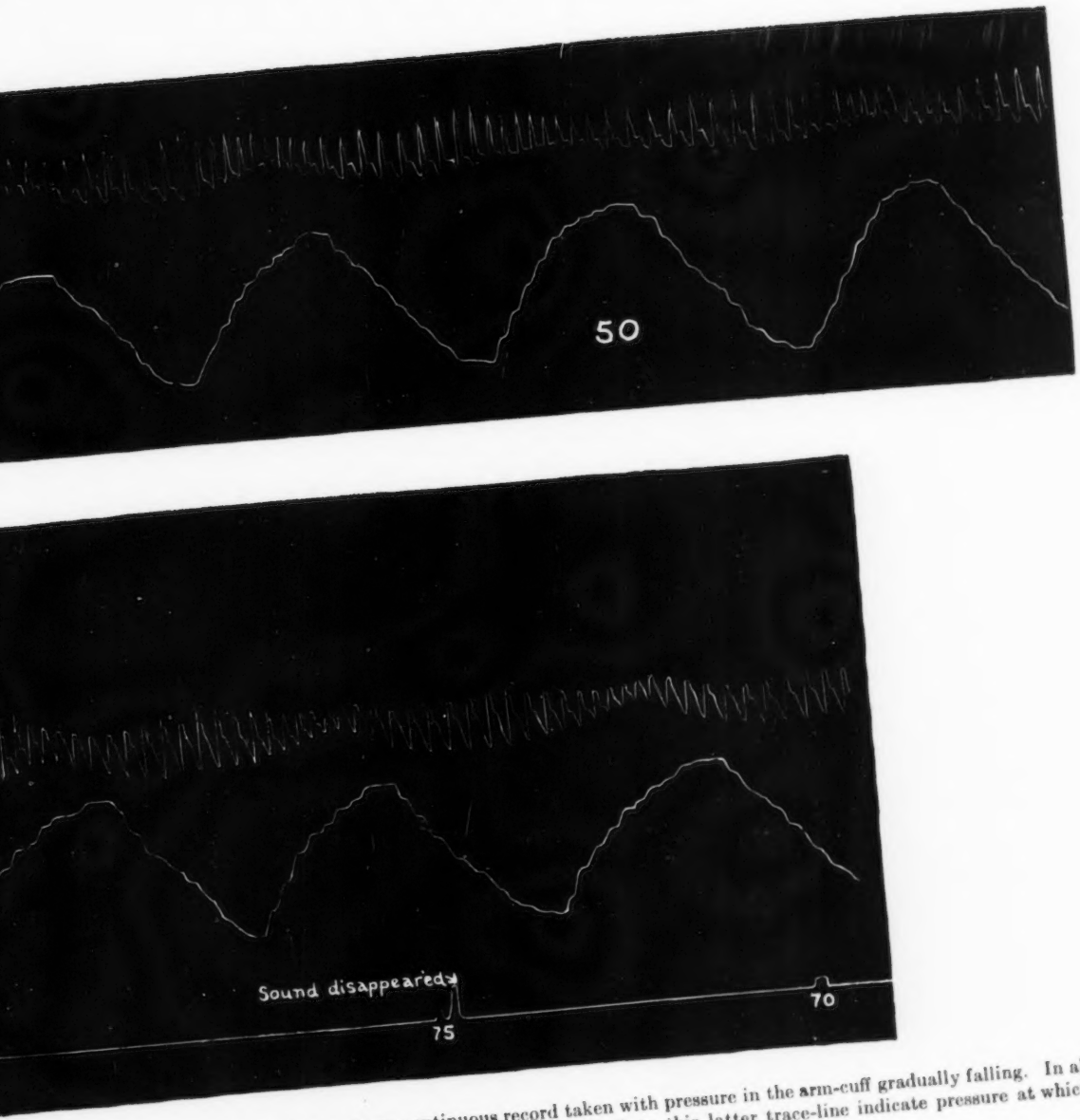


Fig. 4. Subject J. L. S. The experiment was made by Messrs. Rice, Shipton and Stifel. The up
sections of the figure the upper trace-line is that of the respiration lever (the tangent-recording lever in
first and last sounds were heard (auscultatory method).



er sections in the figure are records taken with the extra arterial pressure set at the pressures indicate accompanying numbers. The
this case). The middle trace-line is that of the sphygmomanometer. The lower trace-line is that of a signa key, marking off fall of pressu



mbers. The lower part of the figure shows continuous record taken with pressure in the arm-cuff gradually falling. In all the fall of pressure in 5 mm. intervals. The two irregular signal marks along this latter trace-line indicate pressure at which the

er sections i
this case). T

various points above and below the diastolic pressure, then upon inspection one ought to find an inversion of the respiratory wave in the blood-pressure trace with reference to the waves of the respiration trace itself.

This inversion of the respiratory wave in the sphygmomanometer trace ought to take place in the vicinity of diastolic pressure.

2. Graphic records were taken on twenty-eight individuals, twenty of whom clearly showed inversion of the respiratory wave in the blood-pressure trace. Reproductions of records illustrating the inverted wave are submitted in the report.

3. The inversion of the respiratory wave is not always obtained; eight of the twenty-eight individuals failed to show it. The same individual however who did not show the inversion at one time occasionally was observed to show it at another time. No satisfactory explanation of this failure can at this time be given.

4. It is suggested, and evidence is adduced showing, that the inversion of the respiratory wave in the blood pressure wave may itself at times serve as an additional test in the determination of the correct diastolic pressure in man.

THE TOXICITY OF OIL OF CHENOPODIUM[1]

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Although the oil of chenopodium has been used in medicine more than one hundred years it has as yet hardly attracted the attention of pharmacologists. The first and only record of any experimental studies on animals appeared not quite ten years ago when Brüning (1) published the results he obtained with this drug in different species of animals. His experiments indicate that even in small doses this substance may cause severe symptoms and death. The subcutaneous injection of 0.3 cc. per kilo into rabbits caused death in four days, although 1.5 cc. per kilo, given by mouth, produced salivation only. Two-tenths of a cubic centimeter per kilo, given subcutaneously to dogs, was fatal within twenty-four hours, and 0.2 to 0.4 cc. per kilo produced the same effect in five hours in frogs which received it by injection into the dorsal lymph sac. In the hen, according to his experiments, 0.5 cc. per kilo by mouth produced narcosis, paralysis and death in a few hours. Narcosis was produced in fishes by a concentration of 1:12,500 and death when the concentration was 1:8000. He has shown further, by experiments on frogs, that a more active substance which is toxic also when inhaled, may be obtained from the oil.

In the following experiments it was aimed, as far as possible to determine accurately the resistance of various animals to oil of chenopodium as well as ascaridole (2), which is a peroxide, and a product (3) derived from it, in which the two oxygens were rearranged, transforming it into a dioxide, or glycol anhydride.

[1] The results of some of the experiments were communicated before the American Society of Pharmacology and Experimental Therapeutics. Journal of Pharmacology and Experimental Therapeutics, Vol. II, p. 391.

Attention was also directed to the influence of diet and fasting and to cumulation. A very large number of experiments were performed, but only a few were selected for illustrating the typical action of these substances.

EXPERIMENTS ON RABBITS

The effects of oil of chenopodium and ascaridole, which were given by mouth and subcutaneously, varied a good deal in different individuals, the size of the dose being an important factor in determining the action. After large doses, symptoms appeared within a few minutes even when given by mouth. In some experiments in which a little more than the surely fatal dose was given the effects manifested themselves ten minutes after its introduction into the stomach, but this interval was still shorter when two or three times this amount per kilo of body weight was administered. Symptoms indicating depression of the nervous system usually appeared in one to two hours when given by mouth. The animal became somnolent and inactive, slight incoördination of the muscles of the extremities developing about the same time. Deep coma and paralysis developed later and terminated in death three or six hours after the drug was given. In other experiments tremors and mild spasms appeared first, followed by coma, convulsions and opisthotonus. In a very large proportion of experiments symptoms of a somewhat different order were observed. The initial effects were predominately those of stimulation. Within one to four hours after receiving the oil or ascaridole the animal became restless, looked as if frightened and ran wildly all over the room. Muscle tremors and incoördination became marked and were followed by convulsions which developed within several hours. In some experiments no effects were noticed until the next day. The symptoms persisted several hours and sometimes one, or even two days. The convulsions, which were clonic in character, were sometimes accompanied by peculiar cries, and were sometimes so violent that the cages in which the animals were confined were upset. Dyspnoea, opisthotonus, with cessation of respir-

ation, marked the final stages of intoxication. On autopsy the heart was found beating and continued in this condition several minutes. Although the symptoms of intoxication by the oil of chenopodium and of ascaridole were in most cases practically the same, it was noticed that in a number of experiments convulsions were more apt to follow the administration of large doses of ascaridole. The symptoms produced by the dioxide derived from ascaridole were those wholly due to depression of the nervous system and of the muscles. The reflexes after toxic doses were decreased although the conjunctival reflexes persisted even when the animals were in deep narcosis. Examination of the urine showed a very marked reduction in a number of experiments, but only rarely was albuminuria observed. The appearance of the organs in poisoning with chenopodium or ascaridole indicates that these substances are strong local irritants. The mucous membranes of the stomach and small intestines, when these substances were given by mouth, were inflamed and sometimes hemorrhagic, and the serous coat of the small intestine injected. The kidneys were congested, in rare cases hemorrhagic. The liver in most of the rabbits examined was either normal in appearance or congested. The heart was almost always injected but the lungs were normal in appearance.

The toxicity of the oil of chenopodium varied, though by no means greatly, with the mode of administration. When given by mouth 0.8 to 1.0 cc. per kilo was invariably fatal. Some animals died in four to five hours, although in some cases such a dose proved fatal within one hour. Sometimes the duration of life was longer, but seldom exceeded twenty hours. Smaller doses were rather uncertain in their effects. Thus 0.4 cc. per kilo, when introduced into the stomach of healthy, strong rabbits, produced symptoms of severe intoxication within a few hours in most of our experiments, while the duration of life varied from three and one half hours to three or four days. On the other hand, some individuals survived doses of 0.6 cc. per kilo without showing any symptoms.

The subcutaneous administration of the oil of chenopodium proved to be somewhat more toxic, 0.3 to 0.4 cc. per kilo being

invariably fatal. The appearance of symptoms in these experiments varied considerably. In one experiment marked effects were observed one hour after injection, but more frequently the onset of symptoms was delayed a good deal longer. At least four hours may pass in some individuals after injection without the appearance of any signs of intoxication. This was also observed in experiments with the subcutaneous injection of ascaridole. The duration of life was usually one day, but in some experiments it was only five hours; in others again it was three days. Quantities under 0.3 cc. per kilo were not followed by any symptoms.

Ascaridole, which is the active principle of the oil of chenopodium, was tested in the same way as the oil. Its activity was found to be decidedly greater. One-half of a cubic centimeter per kilo given by mouth produced, in our experiments, violent convulsions, paralysis, and death within one to two hours. Such a dose, it may be observed, was always fatal. A dose of 0.3 cc. per kilo was fatal for some rabbits but was well borne by others. The lethal dose of ascaridole by subcutaneous injection was 0.2 cc. per kilo although some individuals survived the dose. Smaller doses also caused death, but a much larger number survived.

Of considerable interest is the observation that subminimum ineffective doses when repeated one and two, and even three days later, proved to be fatal. This was found to be the case whether the drug was given by mouth or subcutaneously in experiments with the oil, as well as with the active principle, thus indicating cumulative action. The experiments on rabbits which were carried out with the dioxide derived from ascaridole showed that this body was much less active than ascaridole as 0.6 cc. per kilo given by subcutaneous injection failed to produce symptoms. Nine-tenths of a cubic centimeter produced deep narcosis from which the animal recovered, however, in the course of about twenty-four hours.

Rabbit 1593. Belgian Female.
November 21. Weight, 1610 grams.
November 23. Weight, 1665 grams.

9.50 a.m. 1.7 cc. oil chenopodium in water administered by mouth.

10.00 a.m. Somnolent. Ten minutes later symptoms of intoxication well marked. Rabbit comatose and paralyzed.

10.15 a.m. to 10.20 a.m. 16 cc. cottonseed oil given subcutaneously and 22 cc. injected into peritoneal cavity. No convulsions were observed at any time.

12.40 p.m. Died.

Rabbit 1559. Black Female. Weight, 1330 grams.

November 13, 1914. Urine albumin and sugar, negative.

11.12 a.m. 0.8 cc. (0.6 cc. per kilo) oil of chenopodium given by mouth.

12.30 p.m. Muscle tremors.

2.00 p.m. Muscle tremors marked; incoördination.

November 14, 1914. 9.00 a.m. Albumin—none. Reduction—none. Rabbit lying in cage paralyzed.

November 15. Condition worse.

November 16. Dead.

Rabbit 1558. White Female. Weight, 1735 grams.

November 16, 1914. 9.50 a.m. 1.4 cc. oil of chenopodium given by mouth.

1.00 p.m. Mild spasms, muscle tremors and paralysis.

1.30 p.m. Lay in cage as if in comatose condition.

1.50 p.m. Irregular and well marked convulsion preceded by cries. Respiration was suspended about one-quarter of a minute but gradually returned.

2.00 p.m. Convulsions and death. About one minute later thorax opened, heart was still beating but was very weak.

Rabbit 1622. Belgian Female. Good condition. Weight, 2050 grams.

December 14, 1914. 11.00 a.m. Received 0.8 cc. per kilo oil of chenopodium emulsified in 10 cc. saline acacia with a few drops of sodium carbonate. Administered by stomach tube.

12.40 p.m. Muscle tremors very marked, thoracic muscles especially twitching vigorously, but no coma.

1.10 p.m. Muscular twitching more marked.

1.12 p.m. Ataxia of anterior extremities. Rabbit fell over on left side. Head retracted. Spasms of posterior extremities at frequent intervals.

2.30 p.m. Died.

Autopsy: Gastric mucosa slightly hyperemic. Contents smelled of chenopodium. Duodenal mucosa, punctate hemorrhages. Serous coat

injected. Liver congested. Kidney showed hemorrhages of cortex. Bladder distended with urine. Heart injected, in diastole. Lungs inflated but color normal.

Rabbit 542. Belgian Female. Weight, 1725 grams.

December 16. 12.30 p.m. 0.5 cc. oil of chenopodium given subcutaneously. No symptoms observed up to 5 p.m.

December 17. 2.00 p.m. Convulsions. Stood on hind legs and fell over on her back, followed by forced movements. Tremors and restlessness during intervals between convulsions.

4.00 p.m. Quiet.

December 18. 3.00 p.m. Complete paralysis. Passed an enormous amount of feces which was normal in appearance.

December 19. 12.00 noon. Paralyzed and comatose.

Rabbit 615. White and Gray Male. Diet, Oats. Weight, 1420 grams.

May 26, 1911. 11.40 a.m. 0.7 cc. ascaridole administered by mouth.

12.30 p.m. Symptoms appeared—tremors.

1.25 p.m. Coma—opisthotonus. Posterior extremities extended; anterior extremities paralyzed; dyspnoea.

1.30 p.m. Convulsion, accompanied by peculiar cries; marked opisthotonus, and cessation of respiration, but respiration soon returned.

1.40 p.m. Severe convulsions. Rabbit cried and threw itself about on holder. Respiration ceased but soon returned.

1.45 p.m. Violent convulsions; short duration.

1.55 p.m. Violent convulsion; survived.

2.00 p.m. Convulsions with peculiar cries; died in attack.

Rabbit 1561. Gray Male. Diet, Oats. Weight, 1660 grams.

November 12, 1914. Albumin—trace.

1.20 p.m. 0.85 cc. ascaridole given by mouth.

3.00 p.m. Violent convulsions, repeated attacks. No increase of reflexes.

5.00 p.m. Comatose and paralyzed.

November 13. 9.00 a.m. Dead. Urine passed after injection, trace albumin. Reduction heavy.

Autopsy: A few punctate hemorrhages into gastric mucosa which was very pale. Small intestine injected, mucosa red. Kidneys and liver congested.

Rabbit 1549. Belgian Male. Weight, 1000 grams.

November 13, 1914. 2.16 p.m. Injected 1 cc. dioxide derived from ascaridole subcutaneously.

3.00 p.m. Lay in cage unconscious. Reflexes of pupils good.

November 14, 1914. 9.00 p.m. Lay in cage unconscious, apparently comatose, paralyzed. Conjunctival reflexes good, respiration slow.

11.00 a.m. No change.

12.30 p.m. Dead.

Autopsy: Urine in bladder contained a good deal of albumin and showed marked reduction.

EXPERIMENTS ON GUINEA PIGS

The oil of chenopodium, as well as its active principle, ascaridole were employed. The toxic effects first manifested themselves by the appearance of muscle tremors and spasms of the head and neck which were soon followed by mental depression, the animal becoming dull and apathetic. Respiration became slower, and later marked dyspnoea developed. As the stage of intoxication advanced, coma and paralysis were observed Occasionally violent convulsions, almost tetanic in character, were noticed. These were frequently short with intermissions, but in some cases lasted several hours. The time of onset of symptoms usually varied a good deal. It was noticed in a number of experiments that as long as five to six hours elapsed between the subcutaneous administration of the drug and the development of symptoms which persisted at least thirty to forty hours. In other cases severe symptoms developed within two and one-half hours after the same dose per kilo. The toxicity also varied in different individuals. The smallest fatal dose was 0.2 cc. per kilo by subcutaneous injection, the duration of life being less than twenty-four hours; on the other hand larger amounts in proportion to body weight were given without producing any symptoms. The subcutaneous injection of 0.4 cc. per kilo was followed by symptoms within about five hours and death within twenty-four hours, or less. One case may be recorded in which a dose of 0.5 cc. per kilo was survived. The resistance to chenopodium when given by mouth was at least twice as great as when given by subcutaneous injection. Symptoms developed in these experiments approximately three to four hours after the feeding of the drug. One-half of a cubic centimeter per kilo caused death

in two experiments; in one of these when the dose was repeated after an interval of nine days. In three other experiments the introduction of a single dose, 0.5 cc. per kilo, of the oil of chenopodium into the stomach did not produce any symptoms, and the animal survived. A considerable loss of weight, amounting to 15 or 20 per cent, was observed after subminimum doses of chenopodium or ascaridole, whether given by mouth or injected subcutaneously. Ascaridole was found to be much more active than the oil itself. The subcutaneous injection of 0.25–0.27 cc. per kilo was invariably fatal within twenty-four hours. A dose of 0.1 cc. per kilo, given as a 10 per cent solution in neutral olive oil, caused paralysis and coma in twenty-four hours and death in less than forty hours in one experiment. In another experiment such a dose had no visible effect. Oil of chenopodium, from which the ascaridole was obtained, and tested on guinea pigs, failed to produce symptoms in doses of 0.1 cc. per kilo administered subcutaneously. On autopsy signs of irritation of the mucous membrane of the intestine and injection of the blood vessels were very pronounced. The heart was also injected and much darker in color than normal, being almost chocolate colored. This condition was observed in animals to which oil of chenopodium was administered by mouth as well as subcutaneously.

Guinea Pig 192. Male White and Black. Weight, 915 grams.

November 17, 1914. 10.40 a.m. 1 cc. oil of chenopodium administered in olive oil by mouth.

11.15 a.m. No symptoms.

1.40 p.m. No symptoms.

2.00 p.m. Muscle tremors, weakness of extremities.

2.15 p.m. Spasms of muscles of neck. Chin rested on floor. Posterior extremities weak.

3.15 p.m. Coma and paralysis.

4.00 p.m. Dyspnoea, coma, paralysis.

November 18. 9.00 a.m. Found dead.

Guinea Pig 196. Male Black and Yellow. Weight, 955 grams.

November 18, 1914. 11.35 a.m. 5 cc. 10 per cent oil of chenopodium administered in olive oil by mouth.

2.45 p.m. No symptoms.

4.30 p.m. Condition good: No symptoms.

November 19. 9.00 a.m. Alive: No symptoms.

November 27. Condition good.

4.10 p.m. 0.4 cc. oil of chenopodium given by mouth, followed immediately by 10 cc. olive oil.

5.00 p.m. No symptoms.

November 28. 9.00 a.m. Found dead.

Autopsy: Small intestine markedly injected. Mucous membrane inflamed and covered with mucus. Gastric mucosa slightly congested. Considerable oil found in stomach and intestine. Odor of chenopodium very distinct. Heart injected and discolored.

Guinea Pig 197. Male White and Yellow. Weight, 880 grams.

November 18, 1914. 11.00 a.m. 0.5 cc. oil of chenopodium administered by mouth.

2.45 p.m. General tremors, but able to walk well.

4.30 p.m. Weakness of posterior extremities. No other symptoms.

November 19. 9.00 a.m. Found dead.

Autopsy: Findings the same as No. 200.

Guinea Pig 202. Male Black. Diet, Hay and Grass. Weight, 760 grams.

November 19, 1914. 1.59 p.m. 0.8 cc. 10 per cent oil of chenopodium in olive oil administered subcutaneously. Under observation all afternoon. No symptoms.

November 20. No symptoms all day.

November 21. No symptoms.

November 27. Condition good. Weight, 605 grams.

4.05 p.m. 0.35 cc. oil of chenopodium administered by mouth through stomach tube.

December 3. Alive, no symptoms.

Guinea Pig 200. Female Black and Brown. Weight, 755 grams.

November 18, 1914. 12.20 p.m. 3 cc. 10 per cent oil of chenopodium in olive oil administered subcutaneously.

2.45 p.m. Convulsion—frequent attacks and well marked. Short intermission: Able to use legs, that is, able to stand and walk during intervals between attacks.

4.30 p.m. Prolonged convulsions. Attacks every few seconds.

November 19. 9.00 a.m. Found dead.

Autopsy: Heart almost chocolate colored—injected. No odor of chenopodium. Liver congested. Gall bladder distended with clear

bile which was straw colored and odorless. Stomach inflamed in spots. Small intestine much injected and inflamed. Hemorrhagic.

Guinea Pig 195. Male Mole Color and White. Weight, 800 grams.

November 18, 1914. 11.15 a.m. 2 cc. 10 per cent oil of chenopodium in olive oil administered subcutaneously.

2.45 p.m. No symptoms.

3.30 p.m. Paralyzed, but able to crawl with difficulty.

4.30 p.m. Paralyzed, spasms of extremities occurred periodically. Rabbit lay on its side.

November 19. 9.00 a.m. Found dead.

Guinea Pig 198. Male White and Yellow and Brown. Weight, 780 grams.

November 18, 1914. 11.55 a.m. 1.5 cc. oil of chenopodium in olive oil administered subcutaneously.

2.45 p.m. No symptoms.

4.30 p.m. No symptoms.

November 19. 9.00 a.m. Found dead.

EXPERIMENTS ON CATS

When the oil of chenopodium, which was given by mouth and subcutaneously, was administered in sufficient quantity, salivation was the first symptom to appear and frequently lasted until the end of the experiment. Later, depression of the higher nervous centers set in. The animal became somnolent and appeared to be in a semi-conscious condition, even after moderate doses, the head drooping, sometimes the nose touching the floor of the cage, the spine bent and muscular atony being quite marked. Vomiting was observed in a good many cases whether it was given by mouth or subcutaneously, but the time of the occurrence varied. In some subjects it was observed about an hour after the administration, but in others it was delayed for several hours. Incoördination, weakness of extremities, tremors, and convulsions, clonic in character developed in all experiments when the dose was sufficiently large, in which case symptoms of severe intoxication developed within one hour. Convulsions may last one or several days and may become violent. In some experiments convulsions were absent but coma and paralysis developed on the same day, or the day

following the administration of the chenopodium. The resistance in well fed subjects varied a good deal. About 0.6 cc. per kilo of the oil when given by mouth was fatal in every case within eighteen to twenty-four hours. In one cat the duration of life was nearly two days. The administration of 0.4 cc. was followed by symptoms, varying in intensity from somnolence and salivation to violent convulsions and coma. Only one cat survived this dose. Two-tenths to 0.25 cc. of the oil per kilo were toxic; but 0.25 cc. per kilo was fatal in one case. In several experiments, however, the administration of 0.2 cc. per kilo to well fed cats failed to produce any symptoms even when the dose was repeated three days later. When 0.16 to 0.2 cc. oil of chenopodium was fed to cats after they were allowed to fast from four to six days, well marked symptoms developed in all and were fatal in some cases, causing death in less than twenty hours. A second dose given two days after the first, that was only mildly toxic, caused severe symptoms within three hours and the animal died during the night. A smaller dose was not fatal, though one-tenth of a cubic centimeter produced severe symptoms in nearly all of our experiments on starving cats. Attention may be directed, however, to two experiments in which 0.3 cc. per kilo of oil of chenopodium, fed to cats which fasted five days, produced somnolence for about one day, from which they recovered, no other effects having been observed. The drug in this case, however, was given in 5 cc. olive oil instead of being mixed with water or an aqueous solution of gum acacia, as in all the other experiments on cats. As will be seen later, oils and fats probably reduce the toxicity of the chenopodium. That its effects may also be cumulative in cats was also shown in these experiments. The increased susceptibility may last several days as in Experiment 64. The changes observed on post mortem examination were those due entirely to circulatory disturbance. The heart was enlarged and the coronary vessels injected. The kidneys were also enlarged and congested, the liver showed congestion and hyperemia, but this was never very marked. No noteworthy changes could be found either in the stomach or in the intestines.

Cat 63. Tiger Female. Well-fed. Weight, 1950 grams.

December 6. 0.5 cc. oil chenopodium administered by mouth in 5 cc. water.

December 7. Cat vomited during the night; paralyzed; general tremors, coma, reflexes increased. Died in the afternoon of December 7.

Cat 64. Black and White Female. Weight, 2370 grams.

December 6. 0.5 cc. oil of chenopodium administered in 5 cc. water by mouth.

December 7. Cat vomited during the night.

December 12. In good condition. No symptoms. Weight, 2410 grams.

4.00 p.m. 0.5 cc. oil of chenopodium given by mouth in 5 cc. water.

5.00 p.m. Salivation. No other symptoms.

December 13. 9.00 a.m. Lay in cage, paretic.

1.00 p.m. Struggled, attempted to rise. No appetite—refused to eat meat placed before it. Died.

(Note): Cat was fed December 11. No food eaten December 12.

Cat 281. Black Female. Well-fed. Weight, 3200 grams.

November 18. 10.40 a.m. 1.3 cc. oil of chenopodium in water given by mouth.

Symptoms: Mental depression and weakness of posterior extremities appeared a few hours after the oil was given.

November 19. Under observation all day. Frequent attacks of clonic convulsions which became almost violent at times, cat crying frequently during the attacks.

November 20. 9.00 a.m. Convulsions still observed but not so violent. Cat looked less depressed.

November 23. Died about 12 noon.

Autopsy: Cat 281. Liver congested, kidneys enlarged and slightly congested in medulla. Heart congested. Blood vessels all over body injected. No other noteworthy changes.

Cat 282. Female Tiger. Well-fed. Weight, 3075 grams.

November 18. 10.55 a.m. 1.2 cc. oil of chenopodium in water given by stomach tube.

1.25 p.m. Salivation present but no other symptoms.

4.30 p.m. Somnolent. When placed on the floor jumped up suddenly, behaved as if excited.

November 19. Under observation all day. Salivation occasionally. Muscle tremors in posterior extremities, but not paralysis. Condition good.

Cat 279. Black Female. Well-fed. Weight, 2570 grams.

November 17. 11.55 a.m. 0.5 cc. oil of chenopodium in water administered by mouth through stomach tube. Weakness of posterior extremities noticed after several hours.

November 18, 19, 20. No symptoms, appetite good.

November 20. 11.40 a.m. 0.5 cc. oil of chenopodium administered in water by mouth through stomach tube.

3.15 p.m. No symptoms. Survived.

Cat 274. Female Tiger.

November 14. Food withdrawn.

November 16. Weight 1546 grams.

November 18. Weight, 1450 grams.

10.00 a.m. 0.3 oil of chenopodium in water given by mouth through stomach tube.

12.00 p.m. Head bent, drooping, looked sleepy.

November 19. Under observation all day. Somnolent but no other symptoms.

November 20. 9.00 a.m. Condition good, no symptoms.

12.15 a.m. 0.25 cc. oil of chenopodium given by mouth in olive oil.

3.15 p.m. Symptoms marked. Cat cried. Incoördination marked. Refused to get up. Looked severely poisoned.

November 21, 9.00 a.m. Found dead.

Cat 313. Gray and White Female. Weight, 2380 grams.

December 26. 11.30 a.m. Fed 100 grams meat.

1.50 p.m. 0.5 cc. oil of chenopodium injected subcutaneously in back.

3.30 p.m. No symptoms. Animal active and about normal.

3.40 p.m. Vomited and depressed. Lived less than forty-three hours.

EXPERIMENTS ON DOGS

Vomiting, which occurred one to four hours after the administration of the oil of chenopodium, was the first symptom observed when a sufficient quantity was given by mouth or subcutaneously. After large doses, 1.25 to 2.5 cc. per kilo, injected subcutaneously, coma and paralysis also developed, usually within two hours, terminating in death in about six hours. The symptoms were somewhat different after smaller doses. Vomiting and salivation occurred as in the experiments with large

doses, but these were followed by general depression and muscular incoördination. Later, coma and convulsions were also observed and persisted for two or three days. The minimum lethal dose, when given by subcutaneous injection, is about 0.3 to 0.4 cc. per kilo. Smaller doses, 0.2 cc. per kilo, never produced any symptoms; such amounts, however, when repeated after an interval of twenty-four hours, were fatal. That much larger doses may be given by mouth is shown in Experiment 203. It will also be noticed that there was no evidence of cumulative action in this case. Post mortem examination showed the heart to be enlarged and distended, and the coronary vessels injected. There was also enlargement of the kidneys which were congested. The liver was injected and congested, but no other noteworthy changes of the abdominal organs were noticed.

Puppy 204. Weight, 1570 grams.

November 25. 9.39 a.m. 2 cc. oil of chenopodium given subcutaneously.

10.45 a.m. Food vomited; salivation; animal depressed. Able to walk but gait stiff.

4.20 p.m. Dying.

November 26. 10.00 a.m. Found dead.

Puppy 207. Brown Female. Weight, 1550 grams.

November 25. 12.05 p.m. 0.3 cc. oil of chenopodium administered subcutaneously.

4.20 p.m. No symptoms, condition good.

November 26. 10.00 a.m. No symptoms. 0.3 cc. oil of chenopodium administered subcutaneously.

11.00 a.m. Salivation, and animal quiet. Was very noisy before injection.

November 27. 9.00 a.m. Symptoms of severe intoxication; coma and spasms.

November 28. 9.00 a.m. Found dead.

Dog 203. White Female. Weight, 4.4 kilos.

November 21. 1.50 p.m. Received 2 cc. oil of chenopodium by mouth in water by stomach tube.

4.25 p.m. No symptoms.

November 23. 9.00 a.m. Lively, general condition good.

11.50 a.m. Received 2 cc. oil of chenopodium in water and a few drops of olive oil.

12.30 p.m. Food given was vomited. No symptoms developed. Under observation several days.

Dog 212. Black Female. Weight, 10.4 kilos.

December 10, 1914. 10.55 a.m. 3.2 cc. oil of chenopodium injected subcutaneously.

11.15 a.m. No symptoms.

11.40 a.m. No symptoms. Ate meat.

1.00 p.m. No symptoms.

2.00 p.m. No symptoms.

3.00 p.m. Slight incoördination present.

3.05 p.m. Vomited.

4.50 p.m. Somnolent, gait unsteady, but able to walk.

December 11. 9.00 a.m. Looked depressed, laid down and refused to get up, unable apparently to raise herself. Was picked up and made to stand on legs a few seconds; unable to walk, incoördination being very marked in hind legs.

10.30 a.m. Lay in comatose condition; convulsion present.

12.10 a.m. Struggled. Had spasms and cried as if in pain.

December 12. 9.00 a.m. Dead.

Dog 215. Brown Female. Weight, 8.6 kilos.

December 26. 11.30 a.m. Received about 200 grams meat.

1.45 p.m. Received 5.2 cc. oil of chenopodium emulsified with 20 cc. 5 per cent gum acacia and a few drops of sodium carbonate by mouth.

2.15 p.m. Vomited. Salivation. Marked incoördination present. Also narcosis from which dog was aroused with difficulty. Breathing deep, 25 per minute.

4.00 p.m. Deep narcosis. Profuse salivation. Dog seemed to suffer pain. Lived less than forty-three hours.

DISCUSSION

Although the symptoms produced by oil of chenopodium or ascaridole were in the main very similar in all the animals upon which it has been tested, there were nevertheless some differences which may be pointed out with advantage. The guinea pig responded more frequently by showing muscle tremors at first, then convulsions and coma, while in cats and dogs symptoms of depression of the nerve centers were the first effects observed.

In the rabbit the symptoms varied a good deal in different individuals. Narcosis in some and excitement in others were the predominant symptoms observed first. In some animals narcosis was the only symptom when a sufficient amount was administered. In dogs and cats the depressing effect on the nervous system was observed shortly after the subcutaneous administration, followed by deep coma and spasms, which were rather mild by comparison with those in guinea pigs and rabbits. The course of intoxication when the dose was not very large was usually prolonged in all of the animals, lasting in some as long as three and four days.

A comparison of the resistance of different animals shows that the differences are by no means striking, the substance being about equally toxic for the rabbit, guinea pig, and dog, but somewhat smaller doses were required to produce the same effect in cats. As already stated, 0.25 cc. of oil of chenopodium per kilo by mouth produced death in one cat. Approximately twice this dose caused vomiting only in dogs. Such low resistance in the cat may, however, be regarded as exceptional. In most cases 0.4 cc. per kilo may be safely regarded as the lethal dose for the cat, when given by mouth and 0.2 cc. as the fatal dose by subcutaneous injection. Rabbits likewise differed in their behavior toward chenopodium, 0.4 cc. per kilo by mouth being fatal in most cases, but this dose and even 0.6 cc. per kilo were well borne by some individuals. This would indicate that their resistance in general is greater than that of cats. Besides, the dose by subcutaneous injection was distinctly smaller in cats. The toxicity was the same for guinea pigs when they were fed by mouth, but it was distinctly greater when given subcutaneously. It may be remarked in this connection that the difference in resistance when given by mouth and subcutaneously varied considerably in different animals. It will be recalled that in guinea pigs the minimum lethal dose by mouth was fully twice as much as by subcutaneous injection. The case was the same in dogs and in cats, but in rabbits the surely fatal dose by subcutaneous injection was the same as the minimum fatal dose by mouth. This may be explained by as-

suming that the rate of absorption is probably not much different. Indeed, the rapid appearance of symptoms after larger doses when given by mouth indicate that such is probably the case in rabbits, but absorption alone does not explain it, since in the guinea pig symptoms appeared about the same time whether given by mouth or subcutaneously. Some other factors probably enter in the determination of the greater toxicity by subcutaneous injection. May it not be due to detoxication by the intestinal contents? In a number of experiments the overdistention of the gall bladder was noticed at autopsy in the guinea pigs, but not in the rabbits. Perhaps increased secretion of the bile diminishes the toxicity of chenopodium. Detoxication of some of the essential oils by glycuronic acid was claimed by Hildebrandt (7), Matzel (8) and others. Perhaps the oil of chenopodium forms a less toxic compound with the glycuronic acid of the bile.

The effect of the nutritional condition of the animal on the toxicity of chenopodium was very strikingly shown by our experiments on starving cats as four or five days fasting exerted a marked increase in the toxicity of chenopodium. Two-tenths of a cubic centimeter per kilo, administered by mouth, produced death, and 0.1 cc. caused, in nearly all cases, severe symptoms, though recovery was the rule. Poorly nourished animals, such as were frequently brought into the laboratory, which were given chenopodium shortly after their arrival, showed a markedly lower resistance than animals which had been some time in the laboratory and were well fed. The increase of fat in the blood in starvation and the loss of fat from the tissues which occurs in this condition were probably responsible for the decrease of the resistance to chenopodium since a larger amount of it would thus be transported to the central nervous system and other organs instead of being distributed among the lipoids of the body. Support for this view is furnished by experiments we carried out on cats and rabbits in which the effects of various fixed oils on the toxicity of chenopodium were tested.

Examination of the results of the experiments on cats presented in Table 1 shows that in four experiments with 0.4 cc. oil of

TABLE I

Effect of olive oil on toxicity of chenopodium—Experiments on cats

	EXP. NO.	WEIGHT	CHEMOPODIUM PER KILO	SYMPTOMS	DURATION OF LIFE	REMARKS
		grams	*cc.*			
Cats fasted several days before experiment	275	2000	0.3	Slight 2 hours	Survived	15 cc. olive oil given with chenopodium
	278	2100	0.3	Severe	23 hours	Chen. given with water
	290	1710	0.4	1 hour vomiting	4 days	25 cc. olive oil. Vomiting only effect noted.
	291	3035	0.4	Slight	Survived	
	297	4040	0.4	None	Survived	Olive oil 30 cc. with chenopodium
	298	2425	0.4	1 hour severe	About 20 hours	Chen. given in 25 cc. 10% sol. gum acacia
	295	1250	0.4		About 6 hours	Chen. given in 25 cc. gum acacia. Cat emaciated—general condition poor. Coma and conv.
	296	1700	0.4		do	do in olive oil
	292	2465	0.6	Slight	Survived	Chen. in water followed by 30 cc. olive oil.
	289	2010	0.5	1 hour severe	Survived 2 days	Chen. in water followed by 30 cc. olive oil. Coma and paralysis 4½ hrs. Violent conv. next day
	293	3720	0.6	None	2 days	Chen. in 40 cc. olive oil
	294	2925	0.5	1 hour severe.		Chen. in 30 cc. 10% sol. gum acacia. Conv. in 1 hr.

chenopodium, given with olive oil, two survived without showing any symptoms of poisoning. One was found dead four days later but never exhibited any signs of intoxication, while another died after six or seven hours, with typical symptoms of poisoning by oil of chenopodium. This cat, it may be remarked, received chenopodium shortly after it was brought to the laboratory and was emaciated and seemed to be in poor condition generally.

It may be safely concluded, therefore, that such a dose does not produce death when given with fixed oils. It certainly does not cause acute effects, while it will be recalled that 0.4 cc. per kilo fed in water or with acacia was always toxic and was seldom survived. Furthermore, when olive oil was given even a larger dose, 0.6 cc. per kilo, which was invariably fatal when given with acacia or in water, produced in one case slight symptoms only, and the animal survived (292).

Experiments 275 and 278 likewise illustrate the neutralizing effect of olive oil. Both cats were treated in precisely the same way. After having been allowed to fast the same length of time they received 0.3 cc. of the oil of chenopodium by mouth but no effect was noticed as a result of the treatment. A second dose of the same size was given later in 15 cc. olive oil to one and to the other a dose mixed with water which served as a control. As shown in the table, the latter developed symptoms of severe intoxication in two hours and lived twenty-three hours while its mate survived without any appreciable effect.

In experiments on rabbits similar results were obtained. Although the fixed oils failed in some cases to modify the action of chenopodium, the results obtained indicate that the toxicity of chenopodium is undoubtedly decreased by glycerides. As stated above, 0.4 cc. of chenopodium, given by mouth, is often fatal, but some animals, however, may survive as much as 0.6 cc. per kilo. None survived a dose of 0.8 cc. per kilo. Such a dose is, therefore, surely fatal. We found, on the other hand, that 0.4 cc. chenopodium per kilo was never acutely fatal to rabbits which were given cocoanut or cottonseed oil several hours or one day previously. Six-tenths of a cubic centimeter per kilo of oil of chenopodium was fatal to some rabbits but others

survived such a dose. Evidence of detoxicating action was also obtained in experiments with larger doses. It was found that 0.8 to 1.0 cc. per kilo, given in an aqueous solution of acacia as an emulsion caused death in one to five hours. Some animals lived one day. The duration of life in three experiments when the injection of cottonseed oil subcutaneously or into the peritoneal cavity was followed by 0.8 to 1.0 cc. chenopodium, was fourteen to twenty-four hours. In three others similarly treated one cubic centimeter per kilo failed to produce any symptoms in one rabbit, another lived five days, a third, which received 0.8 cc. per kilo, lived nine days. It is evident, therefore, that acute symptoms may be suppressed by simultaneous, or even previous, administration of the fixed oils. It is doubtful whether the fatal outcome may be ascribed to the chenopodium per se. It is more likely that the death was due to loss of appetite produced by it, for in the cats, which did not suffer any loss of appetite, no after effects were observed, while in 290, which refused food, the outcome was fatal. The detoxicating effect of fat is probably due to the solubility of the chenopodium in fixed oils which led Nerking (9) to assume on the basis of the Meyer-Overton theory that anesthetics may be detoxicated by lipoids. He found that if a sufficient amount of lecithin is introduced into the circulation of animals in deep narcosis, the effects of the anesthetic may disappear promptly, also that a larger amount of it is necessary to produce anesthesia if the animal received a previous injection of lecithin. The results of Nerking were disputed by Kramer (10), who repeated the experiment. Our findings, however, would seem rather to support Nerking's conclusions.

Evidence of detoxication by the presence of large amounts of glycogen in the body was also obtained in some of our experiments, as shown in the following abbreviated protocol.

Rabbit 641, diet oats, weight 1375 grams. 0.5 cc. ascaridole per kilo. Found dead three hours later.

Rabbit 615, diet oats, weight 1420 grams. 0.5 cc. ascaridole per kilo. Symptoms appeared within one hour. Died two hours twenty minutes after receiving ascaridole.

Rabbit 640, diet carrots, weight, 1540 grams. 0.5 cc. ascaridole per kilo. Spasms in two hours. Survived.

That the essential oils may be detoxified by glycuronic acid has been shown by Schmiedeberg and Meyer (11), Hildebrandt (12) and later by Matzel (13). Mayer (14) and Hildebrandt (15) found that much larger amounts of glycuronic acid were obtained after feeding grape sugar or cane sugar which might thus explain the effect of chenopodium or ascaridole in starvation. The different behavior of chenopodium in starvation and when administered after feeding a rich carbohydrate diet may be explained therefore by assuming that under these conditions the variation in the amount of glycogen is accompanied by a difference in the quantity of glycuronic acid formed. The formation of glycuronic acid from the glycerin in fats as suggested by Neurberg (16) may also explain in part at least the neutralization of the effects of chenopodium by the glycerides.

Attention has already been called to the cumulative effect of chenopodium. A more complete survey of the data as presented in Table II indicates very clearly that when given even at intervals of several days, symptoms and sometimes death was produced by doses considerably below the minimum toxic amounts. Thus it will be noticed that 0.25 to 0.3 cc. ascaridole per kilo given by mouth caused death after the fifth and even after the fourth dose. When given subcutaneously a non-toxic dose given two or three days later also caused death in rabbits. The experiments in cats have likewise shown unmistakable cumulation. A toxic dose repeated several days later, or even a subminimum dose, if not too small, was fatal when repeated after a few days and four or five small doses administered at intervals of several days were fatal. That this increased susceptibility seems to last for a considerable length of time appears with especial clearness in the guinea pig which received 0.5 cc. oil of chenopodium per kilo in olive oil which failed to cause any symptoms but the same dose was fatal when fed nine days later.

Since the results presented in the present report demonstrate conclusively that chenopodium is a very toxic substance for animals, a word of caution may be addressed to physicians

TABLE II

Cumulative effect of ascaridole and chenopodium

EXP. NO.	WEIGHT	ASCARIDOLE SUBC. INJ.	FIRST DOSE	SECOND DOSE	THIRD DOSE	REMARKS
	grams	*cc. per k.*				
Rabbit 468....	1530	0.22	No symptoms	Fatal		2 doses in 3 days
Rabbit 469....	1075	0.2	No symptoms	Fatal		2 doses in 3 days
Rabbit 473....	840	0.2	No symptoms	Fatal		2 doses in 3 days
Rabbit 479....	790	0.21	No symptoms	No symptoms	Fatal	3 doses in 4 days
		By mouth				
Rabbit 519....	1580	0.3	No symptoms	No symptoms	No symptoms	4 doses in 5 days. 4th fatal
Rabbit 507....	1670	0.3	No symptoms	No symptoms	Fatal	3 doses in 4 days
Rabbit 503....		0.35	No symptoms	Fatal		2 doses in 2 days
		Chenopodium by mouth				
Rabbit 515....	1610	0.6	No symptoms	Fatal in 2 days		2 doses in 3 days
G. P. 196.....	805	0.5				2 doses 9 days apart—duration of life, 1 day.
Cat 278.....	2350	0.3	Slight symptoms	Fatal		Fasting cat—died in less than 20 hours. Chenopodium given in water.
Cat 275.....	2025	0.3	Slight symptoms	Survived		Received 15 cc. olive oil.
Cat 282.....	3075	0.4	Slight symptoms	Fatal		Interval between 1st and 2nd dose 5 days
Cat 280.....	2605	0.2	Slight symptoms	No symptoms	No symptoms	1st, 2d, and 3d doses 3 days apart: 3d and 4th, 2 days. Died after 4th dose
Cat 279.....	2570	0.2	Slight symptoms	No symptoms	No symptoms	Survived 4th dose
Cat 64.....	2410	0.2	Mild symptoms	Died		2d dose 6 days after 1st.

The resistance to ascaridole in these experiments was very unusual. No explanations can be offered at present.

regarding its use in the human subject, more especially as a number of cases of chenopodium poisoning have been reported recently by Levy (17). It is of interest to note that Motter (18) commenting in a recent article in the Public Health Reports on chenopodium poisoning, states that the dose appears to have been excessive and in some cases was repeated, and then quotes several writers who maintained that the use of chenopodium does not cause any important secondary actions.

Since chenopodium is at present being used not only in ascarides but also in hookworm disease which is associated with malnutrition, its exhibition in large and frequently repeated doses cannot be recommended. Brüning (19) and Schuffner and Vervoort (20) recommend as much as one gram every hour until three doses have been given to children from three to thirteen years.

The increased toxicity of chenopodium in starvation and its cumulative effect are important factors as shown in our experiments in determining its toxicity. It is quite possible that the reason that there are so few cases of poisoning in the literature is that castor oil has usually been administered immediately after chenopodium which is quite likely to exert an antidotal influence upon the drug.

SUMMARY AND CONCLUSIONS

Chenopodium produces in cats and dogs, first, symptoms of depression of the higher nerve centers, then convulsions.

The reaction of the rabbit varied, the symptoms being the same as in carnivora in some individuals; in others either excitement or depression only were present.

Guinea pigs presented a picture of poisoning resembling that of rabbits in some respects.

The resistance of dogs, rabbits and guinea pigs to chenopodium was approximately the same. The drug was distinctly more toxic for the cat. The minimum fatal dose, when administered by mouth, was about double that by subcutaneous injection in dogs, cats, and guinea pigs, but the difference was less in the

rabbit. The rate of absorption by subcutaneous injection and by mouth was about the same (as judged by the appearance of symptoms). Ascaridole was about 30 per cent more toxic than chenopodium. Its rearrangement product was less than half as toxic as chenopodium.

The toxicity of chenopodium is distinctly increased in starvation and is decreased by feeding oils and by feeding a rich carbohydrate diet.

Detoxication by glycuronic acid derived from glycogen and glycerides is suggested. The suppression by fixed oils of acute symptoms produced by chenopodium may also be explained by its solubility in oils.

Cumulative effects of ascaridole and of chenopodium were observed in different animals.

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THE ACTION OF GLANDULAR EXTRACTS ON THE SECRETION OF CEREBROSPINAL FLUID

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Introduction. In our previous communications (1, 2), we have shown that an artificial hydrocephalus can be produced experimentally, either by the injection of aleuronant or by blocking the lower end of the aqueduct of Sylvius with gauze or cotton, while a very rapidly fatal hydrocephalus can be produced by a combination of the two methods. We also demonstrated that the cerebrospinal fluid pressure varied with the pressure in the venous sinuses, and that interference with the carotid circulation has only a transitory effect on cerebrospinal pressure and none on the rate of secretion of the choroid plexus. The absorption of cerebrospinal fluid was shown to be largely through the venous channels, 50 per cent to 60 per cent being absorbed in two hours, while the lymphatic absorption is very slow and small in amount. Neutral phenolsulphonephthalein was found to be the best indicator of the rate of absorption from the subarachnoid space.

In the pursuance of the investigations we have had in mind many clinical problems, e.g., meningitis, brain tumors and hydrocephalus, in which the increase in cerebrospinal fluid plays so important a part in the symptomatology and outcome of the disease. Looking upon the choroid plexus as a secretory organ, and confronted with the failure to deal adequately with the overwhelming increase of cerebrospinal fluid by drainage or other means, the question arose in our minds, can the outflow of cerebrospinal fluid be controlled at its source? In thyroid

extract we have discovered a substance with answers this question affirmatively.

This communication is a continuation of our work on the secretion of the cerebrospinal fluid, the main object of which is the determination of such correlations as may exist between the choroid plexus and other glands. In pursuance of this object we have investigated the influence of saline extracts of various organs on the flow of cerebrospinal fluid.

Comparatively little work has been done on the influence of organ extracts on the choroid plexus although the effects of various salts, drugs and other substances have been demonstrated by several workers.

Action of various substances on the flow of cerebrospinal fluid. Cappelleti (3) in 1900 first demonstrated that both ether and pilocarpine increased the flow while atropine slowed it. Dixon and Halliburton (4) in a recent paper confirmed these findings and also demonstrated that brain extract and an extract of the choroid plexus stimulated the secretion of cerebrospinal fluid. Among the various substances, which they tested for possible effect on cerebrospinal fluid secretion, should be mentioned extracts of choroid plexus and of brain, normal salt solution, Ringer's solution, and concentrated solution of various salts; also urea, inosite, peptone, Liebig's extract, pituitary extract, mussel extract, β-iminazolylethylamine, adrenalin, pilocarpine, atropine, cholesterin, cerebrospinal fluid of other animals, and anesthetics, narcotics and hypnotics. None of these gave noticeable changes in the rate of secretion except brain and choroid plexus extracts. They obtained a very slight increase with cholesterin. Pathological cerebrospinal fluid gave interesting results however. The fluid from cases of general paresis, and in one case of delirium tremens, stimulated the flow, while fluid from patients with other diseases did not show any influence.

Methods. As in previous experiments, dogs under morphine-urethane anesthesia were used. The animals were placed in an inclined position on their backs, with the head flexed; a cannula was inserted into the lower end of the fourth ventricle,

which, in this position, is the most dependent part of the cerebrospinal system. The rate of secretion of cerebrospinal fluid was measured by observing the flow into a graduated glass cannula and each 0.01 cc. advance recorded on the drum. Femoral blood pressure and respiratory tracings were made in the usual way and recorded synchronously with the flow of cerebrospinal fluid. In some of our experiments the sinus pressure was also recorded. This was done through a trephine opening into the torcular herophili; in which was inserted a tight fitting cannula connected with manometer filled with a saturated solution of magnesium sulphate. The saline extracts were injected into the exposed femoral vein.

Saline extracts of the following glands were used: brain, thyroid, pancreas, spleen, kidney, liver, testes, and ovary. The glands were removed from freshly killed dogs and ground with clean sand to a fine paste. Normal saline was added in the proportion of 2 cc. to 1 gram of the fresh gland, except the thyroid and adrenals, where 4 cc. to 1 gram were used. This material was then centrifuged at high speed and the supernatant fluid used for the injections. Human thyroids removed at operation from cases of goitre were treated in the same way.

The action of these extracts was controlled by the injection of other substances such as urine, bile, cerebrospinal fluid, chloroform, ether, amyl-nitrate, magnesium sulphate and physiological saline solution.

Experiments. The following records are typical examples of numerous experiments made with each glandular extract. Glands from several dogs were used in order to eliminate any individual variation which might be present.

SPLENIC EXTRACT

Injections of various amounts of splenic extract gave the following results.

1 cc. splenic extract. Normal rate was 0.0375 cc. per minute (0.05 cc. in 82 sec.) After injection of 1 cc. splenic extract the rate increased to 0.0467 cc. per minute (0.2 cc. in 256 sec.)

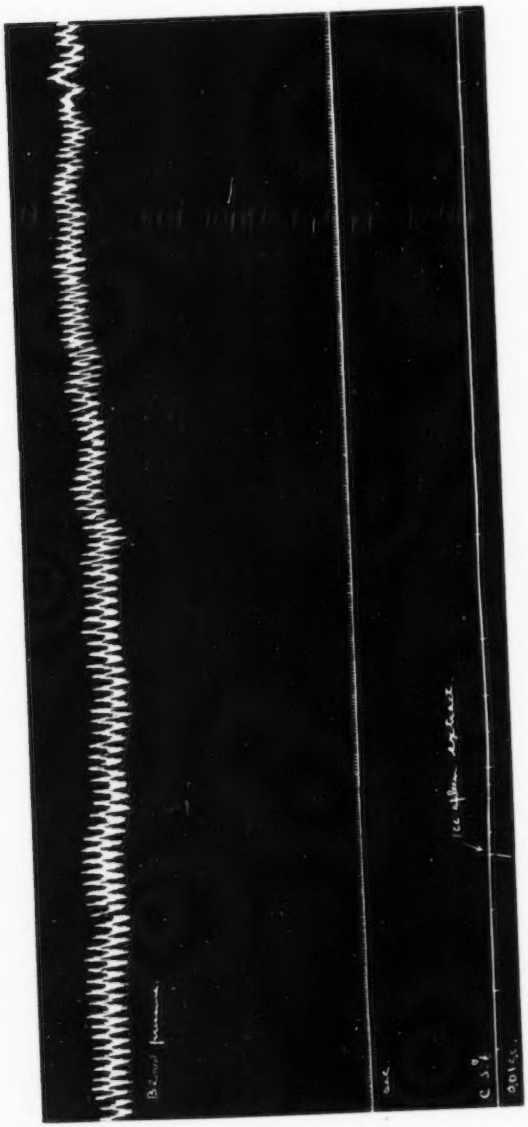

Fig. 1.

or an actual increase of 0.0092 cc. per minute. Very little change in blood pressure resulted. Figure 1.

2 cc. splenic extract. Normal rate was 0.065 cc. per minute (0.13 cc. in 120 sec.) After injection of 2 cc. splenic extract the rate increased to 0.253 cc. per minute (0.43 cc. in 98 sec.) an actual increase in rate of 0.188 cc. per minute. This was followed by an absolute cessation of flow for 197 seconds at which time the flow returned slowly to normal. At the normal rate 0.3195 cc. would have flowed from the cannula during this period. Subtracting this from the actual flow of 0.43 cc. we find there was an actual increase in fluid of 0.1105 cc. above the normal amount for that length of time and an increase in rate of 0.022 cc. per minute. Coincident with the rapid increase in rate a moderate drop in blood pressure was recorded. As the blood pressure began to rise the flow of cerebro-spinal fluid ceased and did not commence until the blood pressure had practically reached normal. The actual rate of flow for the entire period from the time of injection to the return of normal rate is practically normal.

A more marked drop in blood pressure resulted from the injection of the same amount in another dog. In this case the normal rate was 0.0545 cc. per minute (0.06 cc. in 66 sec.). After injection of 2 cc. splenic extract the rate increased to 0.3205 cc. per minute (0.39 cc. in 73 sec.) an increase in rate of 0.266 cc. per minute. A marked drop in blood pressure accompanied this rapid increase in rate. As the blood pressure returned to normal the rate decreased to 0.0162 cc. per minute (0.06 cc. in 222 sec.) a decrease from the normal rate of 0.0383 cc. per minute. The whole period after injection, 295 seconds would yield at the normal rate 0.2679 cc. subtracting this from the actual flow of 0.45 cc. gives an increase in fluid of 0.1821 cc. for the period, and an increase in rate of 0.037 cc. per minute. After the blood pressure had returned to normal, the rate was much slower than before injection so that considering the entire period from the time of injection to the time when a normal rate was resumed we find that practically no actual increase in rate resulted from the injection. Figure 2.

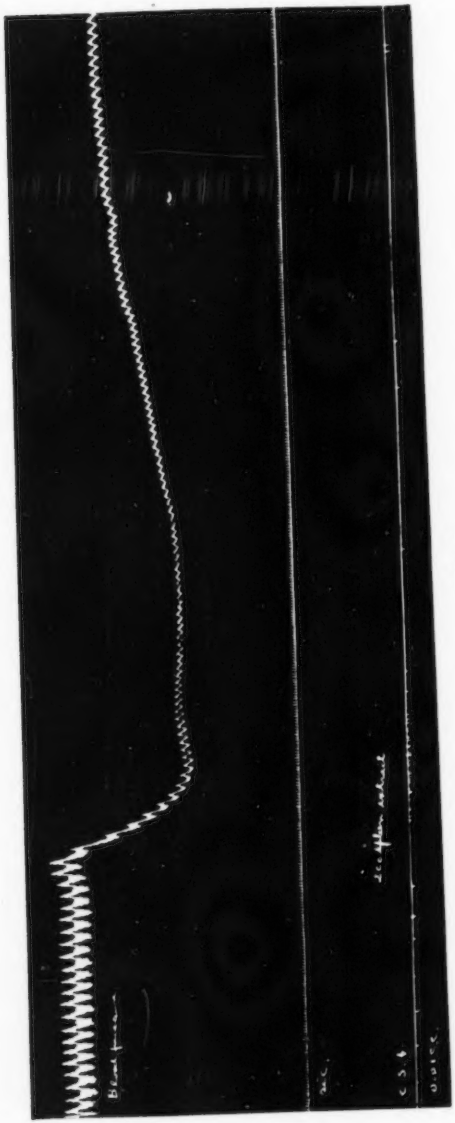

Fig. 2.

4 cc. splenic extract. Normal rate was 0.024 cc. per minute (0.06 cc. in 150 sec.). After injection of 4 cc. splenic extract the rate increased to 0.0986 cc. per minute (0.41 cc. in 249 sec.) an increase in rate of 0.0746 cc. per minute. A steady, though gradual, drop in blood pressure was associated with the increase in rate, which at first was very rapid, but soon decreased to less than normal, so that the final result was the same as before, i.e., practically no increase in rate considering the whole period. Figure 3.

Other splenic injections gave similar results, which may be summarized as a marked drop in blood pressure with a rapid increase in rate of cerebrospinal fluid outflow. The latter was only transitory and was invariably compensated for by the marked decrease in rate or temporary cessation in flow which followed, usually coincident with the gradual return of the blood pressure to normal.

KIDNEY EXTRACT

2 cc. kidney extract. Normal rate was 0.0545 cc. per minute (0.08 cc. in 88 sec.) After injection of 2 cc. kidney extract the rate increased to 0.0634 cc. (0.31 cc. in 293 sec.) per minute, an increase of only 0.0089 cc. per minute. Immediately after injection an irregularity marked by a slight rise and fall in the blood pressure was recorded. This was coincident with the rapid and very transitory increase in rate which was compensated for by a marked decrease in rate immediately following so that the average rate for the whole period is very close to the normal.

2 cc. kidney extract. On this dog the effect of 2 cc. of extract was much more marked than in the previous animal. Normal rate was 0.0818 cc. per minute (0.06 cc. in 44 sec.) after injection the rate increased to 0.415 cc. per minute (1.19 cc. in 172 sec.) an increase in rate of 0.3697 cc. per minute, or over five times the normal rate. Coincident with the rapid outflow was the marked drop in blood pressure. The rate of flow then decreased to 0.066 cc. per minute (0.07 cc. in 63 sec.) and remained slower than normal until the blood pressure returned to its

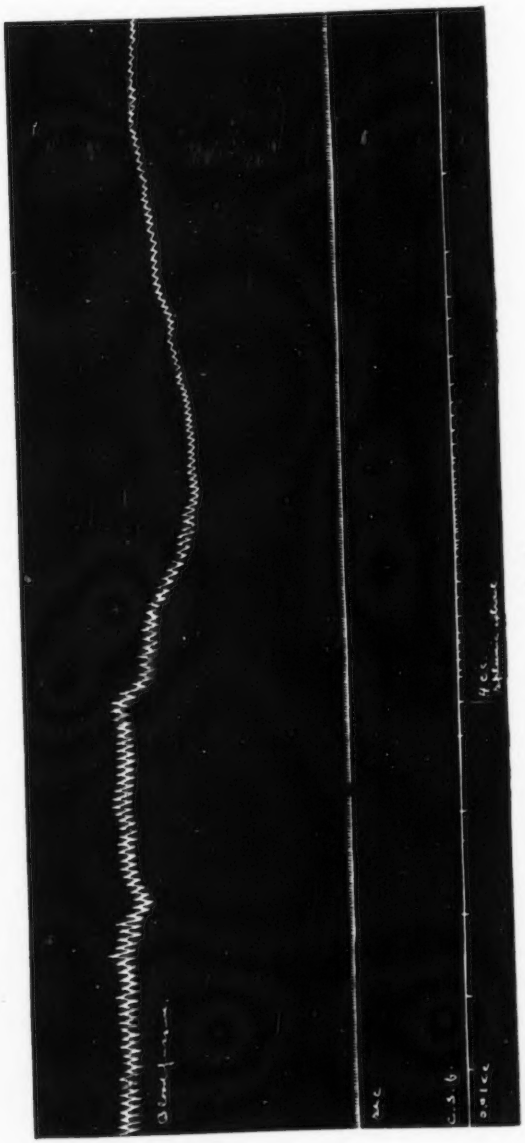

Fig. 3.

former level. The actual rate for the entire period was not increased. Figure 4.

4 cc. kidney extract. Although twice the amount given in the previous experiment was used in this dog the effect was not as pronounced. Normal rate was 0.0499 cc. per minute (0.03 cc. in 44 sec.). After injection of 4 cc. kidney extract a sudden slight drop in blood pressure was recorded and the rate of the cerebrospinal fluid was increased to 0.056 cc. per minute (0.28 cc. in 300 sec.) an increase of 0.0151 cc. per minute. The slight drop in blood pressure was very transitory as it was followed by a rapid return to normal, during which time the flow dropped slightly below the normal rate.

This experiment in connection with those recorded above demonstrates that the amount of kidney extract injected does not directly influence the rate of flow of cerebrospinal fluid, but only has a transitory effect through the fall in blood pressure.

PANCREATIC EXTRACT

The saline extract of pancreas, always gave a marked drop in blood pressure even when comparatively small amounts were injected.

2 cc. pancreatic extract. Normal rate was 0.256 cc. per minute (0.01 cc. in 106 sec.). With the sudden drop in blood pressure the rate increased to 0.56 cc. per minute (0.45 cc. in 50 sec.) an increase of 0.504 cc. per minute. The flow ceased for the next 12 seconds, while in the succeeding 169 seconds 0.2 cc. was drawn back into the cannula. A period of rest of 86 seconds followed before the fluid started flowing at the normal rate. The sucking back of the fluid was probably due to collapse of the cerebral sinuses and was coincident with the gradual rising blood pressure. The very rapid outflow coincident with the drop in blood pressure was probably due to the sudden dilatation of the cerebral sinuses forcing out fluid which had accumulated in the ventricles and possibly the cisterna magna. If we compute the rate of flow for the entire period after injection (0.26 cc. in 317 sec.) we find the rate would be

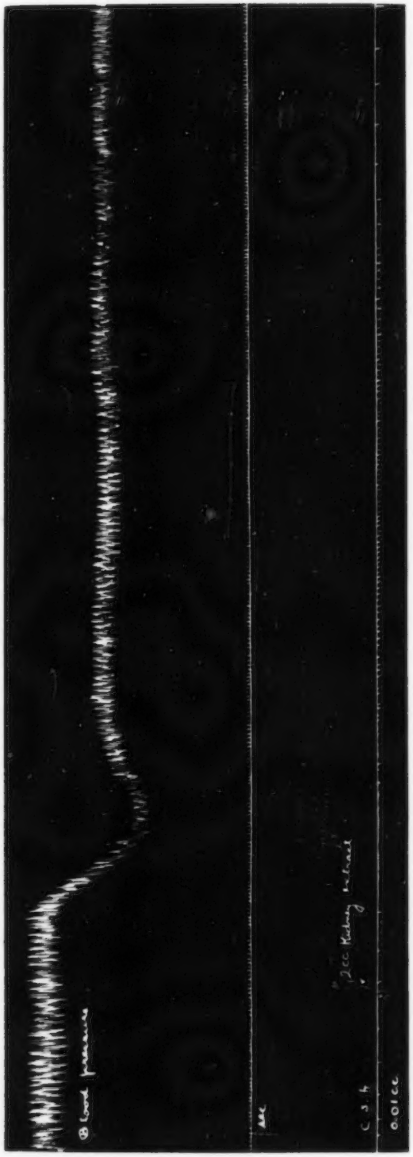

Fig. 4.

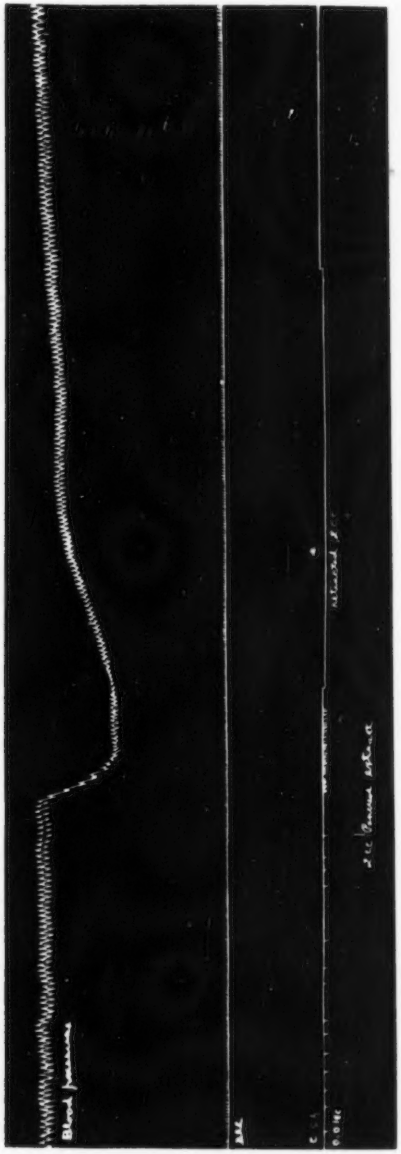

Fig. 5.

0.0492 cc. per minute or a decrease from the normal rate of 0.068 cc. per minute. It is thus seen that while a marked increase in rate of secretion of cerebrospinal fluid followed the injection, this apparent increase was simply the forcing out of fluid already in the cavities of the cranium, by the distention of the sinuses resulting from the sudden fall in blood pressure. The actual rate of secretion was not increased. Figure 5.

A second dog injected with the same amount gave similar results. Normal rate was 0.0671 cc. per minute (0.03 cc. in 134 sec.). After injection of 2 cc. pancreatic extract a sudden drop in blood pressure with rapid rise to normal was recorded. A rapid outflow followed the drop, 0.45 cc. flowing in 78 seconds (0.346 cc. per minute). This was followed by cessation of the flow for the next 138 seconds. The computed rate of cerebrospinal fluid flow for the entire period after injection gives an average rate of 0.127 cc. per minute, an increase of 0.06 cc. per minute or twice the normal rate. The flow was slower than normal for the succeeding three minutes which brings down the average rate per minute for the period after injection to practically normal.

TESTICULAR SECRETION

The normal rate was 0.0574 cc. per minute (0.09 cc. in 94 sec.). After injection of 2 cc. testicular extract there was a slight irregularity in the blood pressure curve and a slight increase in the rate of cerebrospinal fluid flow. This was followed by a slightly slower rate. The rate for the entire period after injection was 0.0636 cc. per minute (0.3 cc. in 283 sec.) an increase over the normal rate of only 0.0062 cc. per minute which is easily within the normal variation in rate. Figure 6.

OVARIAN EXTRACT

Since the ovary consists of such a large amount of connective tissue in proportion to its glandular constituents larger amounts of this extract were used.

Normal rate was 0.0277 cc. per minute (0.05 cc. in 108 sec.). After injection of 5 cc. ovarian extract the rate increased to 0.0391

Fig. 6.

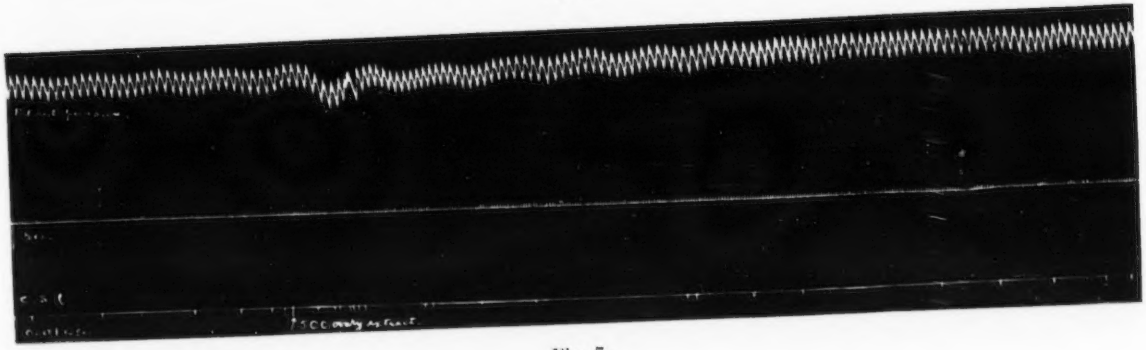

Fig. 7.

cc. per minute (0.22 cc. in 322 sec.) an increase of 0.0114 cc. per minute. This slight increase in rate was due to a sudden short increase in rate coincident with a slight drop in blood pressure. The flow following this period was slower than normal. Figure 7.

LIVER EXTRACT

Comparatively small amounts of this extract cause marked sudden falls in blood pressure, resembling pancreas in this regard.

Normal rate was 0.057 cc. per minute (0.06 cc. in 63 sec.). After injection of 3 cc. liver extract, a sudden, almost vertical drop in blood pressure was recorded and the rate of outflow increased to 0.437 cc. per minute (0.21 cc. in 29 sec.). The blood pressure quickly returned to normal and the rate of outflow dropped to only 0.0108 cc. per minute (0.05 cc. in 276 sec.). The actual rate for the entire period after injection is 0.0509 cc. per minute, which is slightly less than the normal, but within the normal limits of variation. Figure 8.

The experiment with liver extract again demonstrates that while a sudden increase in the rate follows an injection, it is not due to an increase in rate of secretion of the choroid plexus, but is the result of the sudden drop in arterial blood pressure affecting the cerebral sinuses.

BRAIN EXTRACT

5 cc. extract. The normal rate of cerebrospinal fluid flow before injection was 0.0428 cc. per minute (0.09 cc. in 120 sec.). After injection of 5 cc. brain extract the flow increased in rate to 0.286 cc. per minute (0.13 cc. in 90 sec.) and then slowed down to 0.0172 cc. per minute (0.05 cc. in 174 sec.). A slight drop in blood pressure was recorded during which the rate of flow was increased 0.2483 cc. per minute over normal. The actual increase in rate for the entire period after injection was 0.109 cc. per minute (0.48 cc. in 264 sec.). Figure 9.

2 cc. extract. Normal rate before injection was 0.0955 cc. per minute (0.18 cc. in 113 sec.). After injection of 2 cc. brain

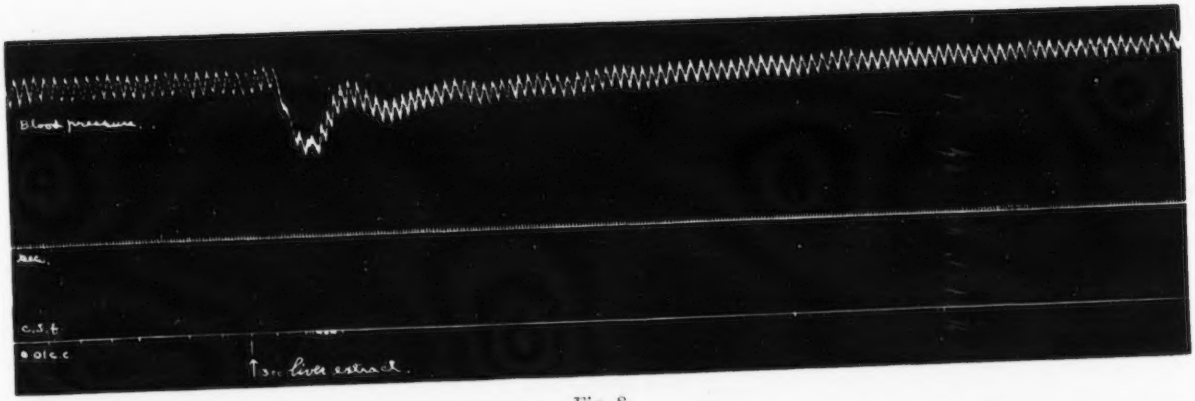
Blood pressure.
Resp.
c.s.f.
0 01 c.c
↑ 5 cc. Liver extract.

Fig. 8.

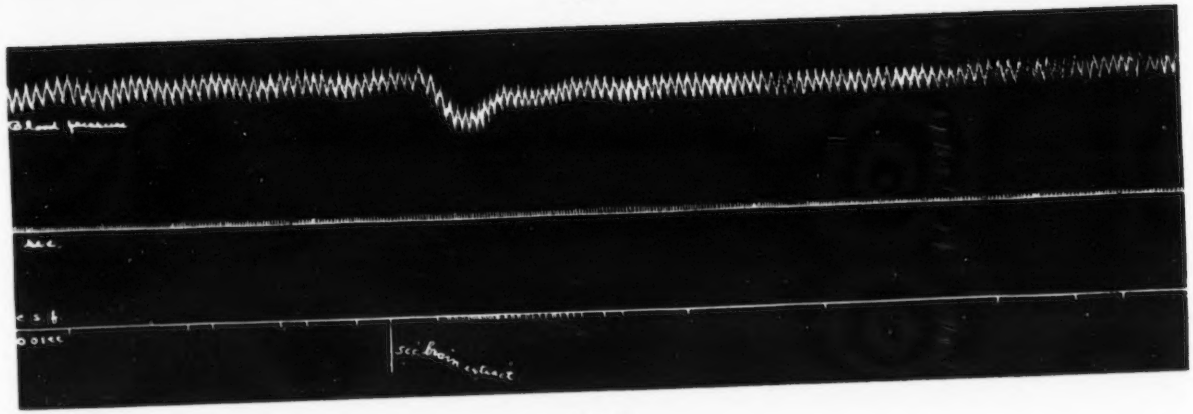
Blood pressure
Resp.
c s f
0 01 cc
5 cc. brain extract

Fig. 9.

extract the rate increased to 0.207 cc. per minute (0.53 cc. in 153 sec.) then ceased for 152 sec. A very slight rise in blood pressure followed by a slight fall occurred. The actual increase in rate for the entire period after injection was 0.104 cc. per minute.

2 cc. extract. Normal rate was 0.0128 cc. per minute (0.08 cc. in 112 sec.). After injection of 2 cc. brain extract a slight fall in blood pressure occurred and the rate of flow of cerebrospinal fluid was increased to 0.135 cc. per minute (0.14 cc. in 209 sec.) or an actual increase in rate of 0.0922 cc. per minute. Figure 10.

5 cc. extract. Normal rate 0.066 cc. per minute (0.07 cc. in 63 sec.) After injection of 5 cc. brain extract a sudden drop in blood pressure was recorded together with a marked increase in rate of cerebrospinal fluid flow. The rate rose to 0.297 cc. per minute (0.42 cc. in 85 sec.). This was followed by the retraction of 0.02 cc. of fluid and a complete cessation in flow for 195 sec. The computed rate for the entire period after injection is 0.0857 cc. per minute an increase of 0.0191 cc. per minute over the normal rate. In this case the marked fall in blood pressure due to the large amount of extract injected apparently has decreased the actual increase in rate which smaller injections invariably show. This cannot be compared with other glandular extracts such as pancreas, liver or spleen, for with these extracts an amount small enough to have very little effect on blood pressure does not increase or decrease even the transitory rate of flow.

The experiments with brain extract demonstrate that there is an actual increase in rate of flow of cerebrospinal fluid independent of the amount of fall in blood pressure. With the injection of the other extracts previously recorded a marked outflow was registered with each drop in blood pressure, but this was invariably followed by a marked slowing or cessation in the outflow or even an actual withdrawal of the fluid back into the cranial cavity. When the rate of flow was computed for the entire period after injection, i.e., the period of rapid outflow plus the period of cessation, the rate was shown to be practically normal, the increase being only an apparent one.

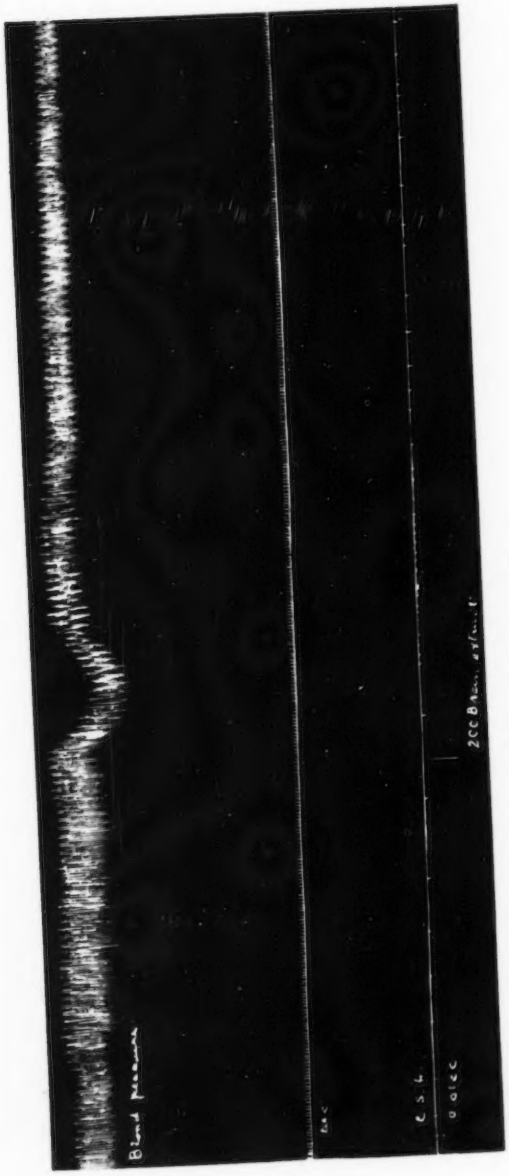

Fig. 10.

THYROID EXTRACT

In our first experiments with thyroid extract we found that the rate of secretion was markedly reduced, therefore the records after injection of thyroid extract were continued over much longer periods (sometimes five or six hours).

5 cc. human thyroid extract. Normal rate was 0.0335 cc. per minute (0.61 cc. in 18 min. 12 sec.). Immediately after injection of 5 cc. thyroid extract there was a moderate drop in blood pressure which persisted for 5 minutes. The rate increased to 0.0714 cc. per minute (0.27 cc. in 3 min. 46 sec.). Following the transitory increase in rate it markedly decreased and after 6 minutes 0.05 cc. of fluid was drawn back into the cannula. The results after injection were computed for 30-minute periods. The rate for the first period was 0.00933 cc. per minute; for the second, 0.00233 cc. per minute; for the third, 0.0103 cc. per minute; for the fourth, 0.0136 cc. per minute; for the fifth 0.0115 cc. per minute. The rate of flow for the whole period after injection, two and a half hours, was 0.0101 cc. per minute, a decrease from the normal rate of 0.0234 cc. per minute. It is thus seen that the normal rate was over three times as rapid as the rate after injection of only 5 cc. thyroid extract.

5 cc. of a 2 per cent. extract of desiccated beef thyroid (commercial). The normal rate of cerebrospinal fluid flow before injection was 0.065 cc. per minute. After injection the rate was slightly increased coincidently with a very slight drop in blood pressure. The results after injection were computed for 30 minute periods as before. The rate for the first period was 0.031 cc. per minute; for the second, 0.0193 cc. per minute; for the third, 0.0153 cc. per minute; for the fourth, 0.0413 cc. per minute; for the fifth 0.023 cc. per minute; for the sixth, 0.0223 cc. per minute; for the seventh, 0.0123 cc. per minute; for the eighth, 0.011 cc. per minute. This gives an average rate for the four hours after injection of 0.0219 cc. per minute. This is a decrease from the normal rate of 0.0431 cc. per minute. As in the previous experiment, the normal rate of secretion of cerebrospinal fluid is three times as fast as after injection of

thyroid extract. It is evident from this experiment that the commercial desiccated thyroid is in every way as efficient in decreasing the secretion of the choroid plexus as is the fresh gland.

3 cc. extract of dog's thyroid. The normal rate of cerebrospinal fluid flow for 30 minutes was 0.04 cc. per minute. After injection of 3 cc. thyroid extract a slight transitory drop in blood pressure was recorded together with a slight temporary increase in the flow of cerebrospinal fluid. The results after injection were computed for 30 minute periods. The rate for the first period was 0.0156 cc. per minute; for the second, 0.0233 cc. per minute; for the third 0.0233 cc. per minute; for the fourth, 0.019 cc. per minute; for the fifth 0.0146 cc. per minute. The average rate for the two and a half hours after injection was 0.0191 cc. per minute. A decrease from the normal rate of 0.021 cc. per minute. At the end of the first 10 minutes in the fourth period 3 cc. of a filtered extract of human goitre was injected without any change in blood pressure or immediate change in rate of secretion, although the rate for the fourth and fifth periods are slightly slower than that for the second and third which may be the result of the second injection.

Physiological saline solution was injected as a control of the saline glandular extracts. The normal rate of flow was 0.026 cc. per minute (0.07 cc. in 161 sec.). After injection of 5 cc. normal saline the rate was 0.026 cc. per minute (0.07 cc. in 160 sec.), exactly the same as before injection. No change in the blood pressure was recorded.

Amyl-nitrate produces results similar in every way to those obtained with organ extracts. In one typical experiment the normal rate of outflow was 0.065 cc. per minute. After inhalation of 5 min. of amyl-nitrate the blood pressure dropped suddenly. The rate of outflow was increased to 0.0341 cc. per minute (0.54 cc. in 95 sec.). This period was followed by a complete cessation of the flow with retraction of 0.09 cc. of fluid as the blood pressure began to rise. The flow then remained stationary for several minutes. The actual flow from the time of injection to time when the normal rate was resumed practically equals the computed normal rate for this period. It is

therefore apparent that the sudden increase in rate was directly associated with the drop in blood pressure as in organ extracts and that no actual increase in secretion resulted.

Similar results were obtained with urine, bile and magnesium sulphate.

SUMMARY AND DISCUSSION

The experiments just submitted illustrate the influence on the rate of outflow of cerebrospinal fluid after the intravenous injection of saline extracts of spleen, kidney, pancreas, testes, ovary, liver, brain and thyroid. A cursory examination of these findings gives the impression of a mass of unrelated, even contradictory facts, *but closer study shows that there are certain phenomena which are common to all these organ extracts, i.e., a more or less marked drop in blood pressure immediately after each injection and a corresponding increase in the rate of cerebrospinal fluid outflow. This depressor effect on the arterial blood pressure is a well known action of nearly all glandular extracts.* The work of Pearce (5), Vincent (6) and many others has demonstrated the constancy of this action and manner of its production. In our work we wish to show the relation which exists between the blood pressure changes and the rate of cerebrospinal fluid outflow after the injection of glandular extracts.

The coincident rapid increase in rate of outflow of cerebrospinal fluid which is invariably associated with this drop in arterial blood pressure has been observed by Dixon and Halliburton (4) and with one glandular extract by Weed and Cushing (7). The former consider the cerebrospinal fluid pressure to be independent of vasomotor changes and therefore conclude that the sudden increase in rate is the result of a hypersecretion of the choroid plexus due to the partial asphyxiation of the secretory cells. Weed on the other hand inclines to the view that the haemodynamic response after injection may account in some cases for the rapid increase in rate.

Our own opinion, which was briefly stated in the Chairman's address before the Section on Surgery of the American Medical Association, June, 1914, *is that the rapid rate of outflow after*

injection of organ extracts is directly due to the drop in blood pressure and not to asphyxiation of the choroid plexus. Our opinion is based upon two distinct but correlated observations. First, a study of the arterial and cerebral sinus pressure before and after the injection. Secondly, a study of the rate of outflow of the cerebrospinal fluid before and after injection.

The haemodynamic influence was studied by tracings taken from the femoral blood pressure together with the record of the coincident changes in the venous pressure in the cerebral sinuses. The latter was obtained by means of a magnesium sulphate manometer connected with a cannula inserted in the torcular herophili. The blood pressure changes were controlled by respiratory tracings, but in our present discussion the latter can be disregarded as under the morphin-urethane anesthesia the respiratory changes are practically negative.

The administration of any depressor substance such as splenic extract, ether, amyl-nitrate, or magnesium sulphate, caused a marked drop in arterial blood pressure followed by a slow rise to normal. Practically coincident with the drop in arterial blood pressure was a sudden rise in the cerebral sinus pressure. This usually occurred immediately after the sudden drop and not with it. The sinus pressure continued to rise as long as the arterial blood pressure remained at its lower level. As the femoral pressure gradually returned to normal the sinus pressure slowly dropped. The outflow of cerebrospinal fluid followed the latter very closely. With each rise of one mm. in the sinus pressure 0.01 cc. or more of cerebrospinal fluid flowed into the graduated cannula. As soon as the sinus pressure dropped the flow of the fluid in the cannula ceased and frequently was drawn back into the ventricles. This demonstrates that the rate of outflow of the cerebrospinal fluid is intimately connected with sudden changes of pressure in the cerebral venous sinuses.

The element of asphyxiation can be ruled out as no change in the respirations occurred. There is no reason to suppose that the choroid plexus is more sensitive to asphyxiation than the respiratory center which certainly would have responded

with increased respiratory movements if even a slight degree of asphyxiation had resulted.

There is one possible complication which should be noted at this time. When ether anesthesia is pushed to the danger point the cerebral sinus pressure rises very high, in fact with a normal of 128 mm. of saturated magnesium sulphate it may rise to 190 or 200 mm. At this time the flow of cerebrospinal fluid is very rapid and more than equals the amount which the ventricles could probably hold. In this case the only explanation which seems feasible is that the very high venous pressure must cause some transudation of fluid through the venous walls. The hypothesis that this enormous increase of fluid is due directly to ether stimulation of the choroid plexus is hardly tenable, since in smaller doses, which do not raise the venous pressure, very little stimulation occurs, certainly nothing which could be compared with the relative size of the dose in the first instance. The possible augmentation of the choroid plexus secretion by the addition of a transudate does not materially affect our former findings, as after the injection of organ extracts no such enormous increase in sinus pressure occurs.

Further proof that the rate of outflow is influenced by the sinus pressure is shown by the reaction to small amounts of depressor substances. An injection of splenic extract, which is sufficiently small not to affect the blood pressure does not cause an increase in the rate of outflow of cerebrospinal fluid.

We are thus led to the conclusion that the sudden increase in rate of outflow following the injection of organ extracts is the result of sinus distension which forces out fluid already present in the ventricles and cisterna.

The second series of observations, which substantiate the opinion that circulatory disturbances are the direct cause of the increased rate of cerebrospinal fluid flow after injections of organ extracts, deal entirely with the rate of outflow and will be considered entirely apart from vasomotor conditions.

The injection of saline extracts of spleen, kidney, pancreas, testes, ovary and liver always results in a temporarily increased rate of cerebrospinal fluid outflow. If the increase has been marked the flow following this transitory increase usually ceases.

A very marked expulsion of fluid is almost invariably followed by retraction of the fluid into the cisterna or ventricles. The comparatively slight increase after ovarian or testicular extract is usually followed by a simple slowing of the rate below normal. The actual rate after injection should therefore be the rate from time of injection to the time when the normal rate was resumed, or at least until the arterial and venous pressure had returned to normal. The rate for the entire period after injection of these five organs was shown to be practically the same as the normal rate before injection. This must of course take into consideration the amount of fluid retracted after the flow had ceased.

Irrespective of the reaction, i.e., whether the flow became subnormal, whether it ceased entirely, or whether it was drawn back into the cranial cavity, no noteworthy deviations from this rule were found.

It was necessary in order to study the flow after the temporary increase in rate to make much longer tracings than are usually made. For this reason we discontinued the use of the smoked paper as shown in our figures and substituted long rolls of paper on which the tracings were made with ink. In this way records of five or six hours could be made without changing the apparatus in any way. We are inclined to think that some of the findings of Dixon and Halliburton are the result of observations over too short periods.

The injection of brain extract gave the same drop in blood pressure and sudden increase in cerebrospinal fluid outflow which was noted with the other glandular extracts. It differs however, from the previous extracts in that the rate for the entire period is greater than normal. In other words brain extract gives an actual increase in the outflow of cerebrospinal fluid. This was determined by subtracting the normal rate from the rate for the entire period after injection (from time of injection to return of normal rate). The average increase in rate of the four experiments quoted is 0.106 cc. per minute.

Thyroid extract gives a decrease in the rate of cerebrospinal fluid outflow, an opposite effect to that of brain extract. With doses of 3 cc. to 5 cc. of thyroid extract the usual drop in blood

pressure with the increased rate of flow of cerebrospinal fluid always occurs. But following this increased rate with its compensatory slower rate or cessation of flow is a prolonged period of markedly decreased rate. This result is obtained by the injection of saline extracts of fresh dog thyroid, fresh human thyroid (colloid goitre), fresh rabbit thyroid and commercial desiccated beef thyroid. The extracts of dog thyroid reduced the flow over one-half during a period of two and a half hours, while extract of human and dessiccated beef thyroid reduced the rate to one-third of the normal, during a period of two and a half hours respectively. Rabbit thyroid injected in doses corresponding to the saline extract of two thyroids (from one rabbit) for a 10 kilo dog gave similar results without the fall in blood pressure, and the resultant increase in cerebrospinal fluid outflow. *In this experiment the decrease in rate appeared almost immediately after the injection and lasted for four and one-half hours at which time the dog was killed.* In similar experiments much longer records have been made, some over a period of five and six hours during which the rate remained much slower than normal. Control experiments have demonstrated that with the methods we used the cerebrospinal fluid will flow at nearly a uniform rate for this length of time.

Further corroborative evidence as to the specific action of thyroid extract on the choroid plexus is furnished by the first clinical case of hydrocephalus in which we tried the effect of feeding dessiccated thyroid. This baby developed hydrocephalus when three weeks old. The thyroid feeding was commenced one month later, at which time the hydrocephalus was marked. Four weeks later the child showed improvement and now after six months has increased one-third in weight, grown four inches taller, and shows no signs of hydrocephalus.

Recently we have carried on a series of experiments with diiodotyrosin which was kindly furnished us by Dr. Treat B. Johnson, who prepared the material. This synthetic substance apparently has an effect similar to thyroid extract although not as marked either in action or duration. A full report this substance will appear in a later paper.

CONCLUSIONS

From the experiments here reported we can state:

(1) Saline extracts of pancreas, spleen, kidney, liver, ovary and testes do not influence the rate of secretion of the choroid plexus.

(2) The apparent increased rate after the injection of extracts of these glands is a mechanical rather than a secretory effect.

(3) This mechanical effect is directly due to the fall in arterial blood pressure which increases the pressure in the cranial venous sinuses thus forcing out the preformed fluid in the ventricles and cisterna magna.

(4) Brain extract causes an increase in secretion independent of blood pressure changes.

(5) Thyroid extract, either from fresh glands or the commercial desiccated beef preparation is the only glandular substance which has a specific inhibitory effect on the secretory activity of the choroid plexus, quite independently of blood pressure changes.

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SOME METABOLIC INFLUENCES OF BATHING IN THE GREAT SALT LAKE

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The value of regular and systematic bathing as a health-producing agency is most emphasized by those who make periodical visits to bathing resorts and by the physicians under whose direction they live while there. An explanation for the salutary effect of the water is usually that it "stimulates metabolism." Whatever influence the bathing may have, the effects of simple food, regularity, new surroundings, and the careful attention of physicians to all ailments, small and large, are factors, which, according to Umber (1) and others (2) are more favorable, therapeutically, than any constituents of the water. The desirable results of the use of large amounts of water internally, which is usually a part of the régime, also come into consideration (3) (4).

According to Matthes (5) in v. Noorden's treatise on metabolism the effects of baths and bathing may be credited either to the temperature of the water or to its mechanical or chemical action.

With respect to the influence of temperature, both cold and hot baths may bring about an increased metabolic exchange, the combustion of non-nitrogenous substances being first affected. If material disturbance in the heat balance occurs, protein metabolism may also appear to be influenced. Little is known regarding the individual nitrogen components. Formanek (6) investigated the influence of cold baths on total nitrogen and uric acid excretion in a human subject on a uniform mixed diet and found an increase in both, with an increased fecal nitrogen

at the same time, indicating poorer utilization. In similar experiments on a fasting dog (7) in which the body temperature fell to 29.3° the nitrogen excretion rose from 1.4 to 4.6 grams per day and remained at this high level for several days. In a hot bath Rubner (8) found metabolism stimulated, as measured by respiratory exchange. If the heat is sufficient to raise the body temperature considerably, there is an increased protein metabolism, not, according to v. Noorden, as a primary result of increased body heat, but after increased carbohydrate metabolism has depleted the glycogen stores. A possible direct influence can hardly be excluded. A stimulation of protein metabolism as the result of hot baths has been observed by several workers (9) (10) (11).

The metabolic influences of different substances in the bathing water has been looked for chiefly in brine baths of various concentrations, in sea water baths, in sool baths (Stassfurt salt) with and without carbon dioxide, in mustard baths, in those containing radium emanation, and in many others.

(a) *Protein metabolism.* The work of earlier investigators is conflicting, indicating in some cases an increased, in others a decreased, nitrogen excretion following brine and sool baths. Bahrmann and Kochmann (2) question these results because the preliminary periods were generally too short to observe nitrogen metabolism properly. Also the nitrogen in feces and sweat is not accounted for. They conclude that the effect of sool baths on metabolism is no different from that of ordinary baths at the same temperature. In experiments on two strumous children, five and eight years old, Heubner (12) found increased nitrogen excretion during a bathing period in which the baths, beginning with pure warm water, were next 3 per cent and then 5 to 7 per cent NaCl. The high nitrogen excretion continued through the final period. The calorific value of the diets was the same, and the nitrogen content almost identical, yet the increased nitrogen elimination produced a minus balance in the less well nourished of the two children throughout the latter part of the bathing period and the final period.

(b) *Metabolism of sodium chloride.* Regarding variations in

sodium chloride excretion the findings are also contradictory, and Bahrmann and Kochmann (2) are unwilling to use most of the data because in some cases the analytical methods were not the best, and because the laws of sodium chloride metabolism are not well enough understood. That variations in the amount of salt excreted are at least not to be explained by absorption of salt through the skin is generally accepted. Heubner (12) found a retention of NaCl in the poorly nourished subject during the bathing period, while in the well-nourished the NaCl elimination was uniform throughout.

That the salts of a bath may remain on the skin a considerable time after the bath if not rinsed off with fresh water was shown by Lehmann (13) who was able to demonstrate spectroscopically the presence of lithium and strontium on the skin a week after a bath containing these salts, which may therefore continue to exercise an effect on the skin (14), if, indeed, they have an effect. Several ingenious suggestions are made, that the salt on the skin makes a cloak, lessening the heat loss through evaporation (15), or that the water taken up by the hygroscopic salts stimulates the skin and increases oxidation (16).

(c) *Gaseous metabolism.* Here again the early observations are contradictory, but the opinion of Winternitz (17) that sool baths, even 15 per cent salt, have no other effect than sweet water baths of the same temperature is no longer questioned. In the extensive work on the influence of sea climate and bathing on man Loewy and his co-workers (18) found an increased oxygen consumption. During the first few days a decrease in respiratory quotient was noted. Incidentally also, they found nitrogen metabolism increased in one individual, decreased in another, and during the bathing period a sodium chloride excretion greater than the ingestion. The effect of a sea bath, they state, is entirely different from that of a cold tub, and the unknown factors in this explanation probably account for the many conflicting results.

The Great Salt Lake offers an unusual salt concentration. Because of this and because of the beneficial influences that are claimed for its water, it seemed desirable to learn whether bath-

ing in this water modified metabolic processes in any tangible way.[1]

Two subjects were maintained on a uniform diet as follows:

	Subject 1	*Subject 2*
Graham crackers, per meal	140.0 g.	110.0 g.
Peanut butter, per meal	20.0	20.0
Butter, per meal	25.0	25.0
Milk, per meal	425.0 cc.	400.0 cc.
Water, per day	1200.0 cc.	600.0 cc.
Tea (evening), per day		300.0 cc.
Nitrogen per day	14.0 g.	12.6 g.
Calories per day	3380.0	2962.0

The experiment was divided into five periods: (1) a preliminary period of five days, (2) a bathing period of four days, (3) two days without bathing, (4) four days of bathing, (5) a final period of two days. Periods 2, 3 and 4 are sometimes referred to as the bathing period. The daily routine of the subjects was practically uniform throughout the experiment. During the bathing days the laboratory work was augmented by a 15-mile ride to Saltair and back, and by the usual exercise while in the water. No definite activity was substituted for this during periods 1, 3 and 5.

The composition of the water of Great Salt Lake has been the subject of several inquiries (19), and has been found to vary considerably with the seasons and with the amount of rainfall. Generally speaking the water shows a minimum of total solids after the spring thaws, the amount increasing through the dry summer months and coming to a maximum in the fall before the winter precipitation begins. Analysis of a sample taken during the bathing showed a specific gravity of 1.152, 19.17 per cent total solids, of which 93.34 per cent were chlorides calculated as NaCl. After the bath the face and hands were rinsed with fresh water, the rest of the body being wiped immediately with a dry towel. The original plan had been to make the bath during period 2 from twenty to thirty minutes long, and during period 4 from forty-five to sixty minutes. Ordinarily the weather in Salt Lake is warm enough, by the end of June, to make this possible, but a week of cold,

[1] It is a pleasure to acknowledge the assistance of Saltair Beach Company, who provided transportation and the privileges of their bathing equipment.

rainy weather at the end of the experiment made it quite impossible to do this and still have anything like normal bathing. The local weather bureau records a higher precipitation (3.37) for the month of June 1913, than has occurred in any June since the local weather bureau was established in 1874. The excess over the normal was 2.6, and practically all of this precipitation came during the days of the second bathing period.

The urine was collected in 24-hour samples, preserved by thymol, and cold, and analyzed in duplicate for total nitrogen (Kjeldahl), creatinine (Folin), chlorides (Dehn-Clark) and uric acid (Folin-Macallum).

Acidity (Folin) was determined during the latter part of the experiment, but the results are not significant; ammonia was determined throughout, but the discovery, later, that the distilled water was untrustworthy caused us to question these results, even though duplicate analyses were obtained. This also necessitated a repetition of the total nitrogen determinations. The original plan of the work had included the determination of fecal nitrogen and chlorides, and of urinary indican, and observations of blood pressure and blood count as affected by the bath, but these had to be relinquished before any conclusive results were obtained because of the inadequacy of the working force. The omission of blood pressure determinations is particularly unfortunate because, in view of certain clinical results, the pressure of the heavy water on the exterior of the body may materially increase blood pressure, and as a result, perhaps modify metabolic processes in the tissues.

The results of the urine analysis, and a record of the length and temperature of the baths are shown in Table 1. Figure 1 shows graphically the variations in the urine constituents during the experiment.

Total nitrogen. The results indicate a slightly increased nitrogen elimination during the bathing period, 7.16 per cent above the average of the non-bathing days in Subject 1, where the variations follow the periods rather closely. In Subject 2 the effect is not as clear, though periods 2 and 4 together average 5 per cent greater nitrogen excretion than the average for all non-bathing periods. The fact that nitrogen elimination in

period 3 (two days without bathing) is higher than in period 2 and almost as high as in period 4 may be due to a lag which is noted in much of the data for Subject 2. The fall in body temperature during and following the baths (p. 000) was small and

TABLE I.

Subject 1

DAY OF EXPERIMENT	DATE	BODY WT.	URINE VOLUME	TOTAL N	URIC ACID	CREATININE	CHLORIDES	TEMP. OF AIR	TEMP. OF WATER	LENGTH OF BATH MINUTES
		Pounds	*c.c.*	*Grams*	*Grams*	*Grams*	*Grams*	*Cent.*	*Cent.*	
1	6/13	131.4	1722	11.72	0.38	1.24	5.87			
2	14	131.5	805	10.67	0.36	1.16	4.80			
3	15	131.6	1130	11.84	0.37	1.02	5.44			
4	16	131.8	1030	12.04	0.33	1.28	6.27			
5	17	132.0	1725	12.15	0.28	1.24	6.70			
Average.				**11.68**	**0.34**	**1.19**	**5.82**			
6	18	132.5	1558	11.98	0.31	1.19	7.91	26.0	22.2	15
7	19	132.3	1265	11.84	0.33	1.28	7.76	31.0	23.6	30
8	20	131.7	1448	12.35	0.29	1.26	7.05	23.5	23.0	20
9	21	131.9	1162	12.21	0.32	1.16	6.01	27.0	24.5	30
Average.				**12.10**	**0.31**	**1.22**	**7.18**			
10	22	132.3	870	11.22	0.23	1.13	5.64			
11	23	132.7	1650	12.64	0.31	1.24	7.15			
Average.				**11.93**	**0.27**	**1.19**	**6.40**			
12	24	133.3	2110	14.50	0.25?	1.21	7.91	21.8	22.8	45
13	25	133.3	1860	13.16	0.26	1.31	8.84	20.5	22.5	30
14	26	132.3	1305	12.70	0.28	1.22	7.54	17.5	21.0	20
15	27	133.0	1475	11.89	0.27	1.20	7.48	20.5	20.5	15
Average.				**13.06**	**0.27**	**1.24**	**7.94**			
Average.	18-27			**12.45**	**0.29**	**1.22**	**7.33**			
16	28	133.3	1350	11.75	0.31	1.22	6.64			
17	29	133.3	1278	11.25	0.31	1.10	6.70			
Average.				**11.50**	**0.31**	**1.16**	**6.67**			

brief. The calorific value of the food as well as the constancy in body weight would suggest that the slightly higher excretion of nitrogen during the bathing periods is a result of stimulated metabolism, rather than a utilization of proteins to meet an increased need for fuel.

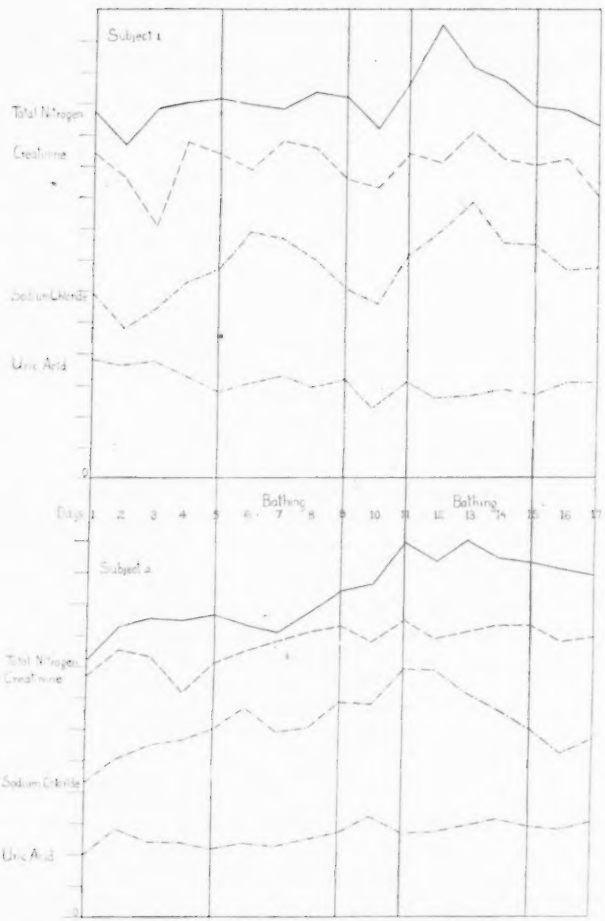

Fig. 1. Total nitrogen and chlorides are plotted in grams, uric acid and creatinine in tenths of grams, all to the same base line for each subject except creatinine in subject 1 which is dropped two spaces to avoid confusion.

Uric acid. The diet was practically purine-free, so that the uric acid may be considered as endogenous in origin. The variations in its elimination are too slight to indicate any effect of the bathing; to be complete these results should be supplemented by values for uric acid in blood which was not determined.

Subject 2

DAY OF EXPERIMENT	DATE	BODY WT.	URINE VOLUME	TOTAL N	URIC ACID	CREATININE	CHLORIDES	TEMP. OF AIR	TEMP. OF WATER	LENGTH OF BATH MINUTES
		Pounds	*c.c.*	*Grams*	*Grams*	*Grams*	*Grams*	*Cent.*	*Cent.*	
1	6/13	103.7	1238	8.21	0.20	0.77	4.31			
2	14	104.1	1177	9.27	0.28	0.85	5.07			
3	15	104.5	1288	9.54	0.24	0.83	5.51			
4	16	104.9	1148	9.45	0.24	0.72	5.66			
5	17	104.9	1318	9.63	0.22	0.81	6.01			
Average.				**9.22**	**0.24**	**0.80**	**5.31**			
6	18	106.0	1383	9.33	0.24	0.85	6.68	26.0	22.2	15
7	19	106.0	1207	9.08	0.23	0.88	5.94	31.0	23.6	25
8	20	106.3	1314	9.73	0.25	0.91	6.05	23.5	23.0	15
9	21	106.7	1471	10.43	0.27	0.93	6.87	27.0	24.5	30
Average.				**9.64**	**0.25**	**0.89**	**6.39**			
10	22	106.5	1130	10.66	0.32	0.88	6.82			
11	23	106.8	2015	11.97	0.27	0.95	7.95			
Average.				**11.32**	**0.30**	**0.91**	**7.39**			
12	24	106.5	1647	11.37	0.27	0.89	7.91	21.8	22.8	40
13	25	106.5	1600	12.03	0.29	0.91	7.17	20.5	22.5	25
14	26	106.4	1446	11.47	0.31	0.93	6.61	17.5	21.0	15
15	27	106.5	1343	11.31	0.29	0.93	6.01	20.5	20.5	10
Average.				**11.55**	**0.29**	**0.92**	**6.93**			
Average.	18-27			**10.74**	**0.27**	**0.91**	**6.80**			
16	28	106.7	1190	11.09	0.28	0.88	5.24			
17	29	107.1	1330	10.89	0.30	0.89	5.65			
Average				**10.99**	**0.29**	**0.89**	**5.45**			

Chlorides. An increased elimination of chlorides during the bathing period is evident, amounting to 20.95 per cent for Subject 1, and 27.10 per cent for Subject 2, over the average amounts eliminated during the preliminary and final periods. In Subject 1 these variations again follow the separate bathing

periods closely, while in Subject 2 the two-day interim without bathing shows a higher excretion than the bathing periods, both of which, however, show markedly higher values than preliminary and final periods. Regarding the absorptive powers of the human skin a considerable body of literature over long years fails to give conclusive evidence. It is generally held that watery solutions, not acting chemically on the epidermis, are not capable of absorption by the intact skin of man, while such fluids as can wet the skin, namely, fats and their solvents, may be imbibed by the cells, or may make their entrance through the capillary spaces between them (20). That the sebum is in fact the barrier to the entrance of aqueous solutions is made probable by the fact that after cleansing the skin with ether it is no longer impermeable to such solutions. Of particular interest are the results of Kahlenberg (21) who was able to show the rapid absorption of boric acid through the ether-cleansed skin of the feet. It is probable that the capillary spaces as well as the cells themselves may serve as channels for absorption, since rubbing on various ointments is more effective as far as absorption is concerned if the skin is rubbed in one direction only. Mucous surfaces did not furnish an avenue of entrance as special care was taken to avoid getting water into the mouth. As the skin was not rinsed off with fresh water following these brine baths considerable salt remained as a thin film and it is possible that, aided by the friction of the clothing, the very small salt particles might gradually make their way between the capillary spaces and so come to absorption.

To say that all of the augmented elimination of chloride came from salt absorbed through the skin would be to leave out of account the considerable stores of chloride over which the body has disposition. Such stores are made evident at the beginning of a fast when the chloride excretion for the first day or two is much above the level to which it later falls (22). The variations in this experiment might be considered as expressive of an increased catabolism of chloride-containing compounds. This increase is not shared by any other urinary constituent determined except total nitrogen; the parallelism in the total nitrogen and chloride curves is especially pronounced in Subject 1,

but the increase in protein catabolism is entirely inadequate to account for the increased chloride excretion (23). Further, it is usual in metabolism experiments to find chloride and nitrogen entirely independent of each other. If the parallelism is not accidental we have no sufficient explanation for it at present.

Creatinine. The creatinine variations, while very slight indeed, are at least consistent in showing increased excretion during the bathing periods, though here again Subject 2 shows a lag. Creatinine, as an end product of endogenous metabolism is generally unaffected, as to its amount, by muscular activity; muscular tonus, on the other hand, has been thought to be more closely related (24), and if this is so, and if the values obtained were significant, the question would be answered. Subsequent work during bathing in colder water[2] failed to support this idea.

[2] Creatinine determinations were made during a stay at the sea-shore; the water was considerably colder (15°C.) and the salt concentration, of course, much less. For reasons of convenience the subjects were not placed on a uniform diet, but since creatinine excretion is independent of all dietary constituents except the creatinine of meat, and since, on an ordinary uncontrolled meat diet the average variation from the non-meat diet is very slight, 0.05 gram (Shaffer, Am. J. Physiol., 22, 1908, 454), it was thought not necessary, inasmuch as any variations, in order to be attributed to bathing would have to be greater than those that might result from variations in a partially controlled diet. The results are given in the subjoined table, and show no more evidence of increased creatinine excretion following bathing than do the data of Table 1. The constancy of creatinine excretion even on a mixed diet is noteworthy.

	SUBJECT 1		SUBJECT 2	
Date	*Creatinine grams*	*Length of bath*	*Creatinine grams*	*Length of bath*
July 23	1.56		1.03	
July 24	1.50		1.14	
July 25	1.37		0.98	
July 26	1.48		0.96	
July 27	1.50		1.04	
July 28	1.48		0.98	
July 29	1.47	20 minutes	1.00	15 minutes
July 30	1.38	no bath	0.98	no bath
July 31	1.49	22 minutes	1.07	15 minutes
August 1	1.55	27 minutes	1.06	20 minutes
August 2	1.40	20 minutes	1.04	20 minutes
Average				
Non-bathing days	1.47		1.02	
Bathing days	1.48		1.04	

The higher values in this table as compared with Table 1 are not significant. The dichromate used as a standard, even though taken from a paraffined bottle, contained moisture which was not removed.

The examination of hourly samples might reveal variations which 24-hour samples do not show. Perhaps the great lowering of body temperature frequently produced experimentally in man and animals by exposure to cold might modify creatinine metabolism. So far as we are aware, no work has been published, showing the effects of lowered body temperature on creatinine excretion, although its variations in fevers and other pathological conditions are known.

TABLE 2

DATE	TIME	TEMPERATURE (FAHRENHEIT)		REMARKS
		Subject 1	Subject 2	
June 19.....	4.20 p.m.	99.6	99.6	before bath
	4.50 p.m.	99.1	98.4	just after bath
	7.00 p.m.	99.4	99.5	after return to laboratory
June 21.....	1.35 p.m.	99.35	99.6	before lunch
	3.25 p.m.	99.4	99.75	before bath
	3.55 p.m.	99.15	98.3	immediately after bath
			98.0	3 minutes later
		98.4	97.95	10 minutes later
		99.2	99.7	after return to laboratory
June 24.....	3.45 p.m.	99.62	99.95	before bath
	4.35 p.m.	99.2	97.3	5 minutes after bath
			98.0	8 minutes after bath
		98.8	98.0	10 minutes after bath
		98.5		15 minutes after bath
June 25.....	3.40 p.m.	99.55	99.85	before bath
	4.15 p.m.	99.6	98.5	5 minutes after bath
		99.2	98.5	8 minutes after bath
		98.9		11 minutes after bath
		98.85		14 minutes after bath

Body temperature. The drop in temperature resulting from the bath was usually about 1° from Subject 1, and nearly 2° for Subject 2. Readings were taken only occasionally and while the temperature changes are not great some interest attaches to the measurements which are of the rectal temperature. Some of the results are given in Table 2.

Although the subjects stayed in the water until uncomfortably cold, the body temperatures immediately after bathing are

generally not the lowest reached, but the temperature continues to fall (Subject 1) for five to ten minutes, while the body is being dried with a towel and the usual clothing is being put on. This, we find, has been noted before (25). Probably the evaporation of such moisture as is not removed by drying with a towel is sufficient to account for this fall in body temperature.

SUMMARY

Two subjects were maintained on a uniform diet containing in one case 14.0, in the other 12.6 grams of nitrogen daily for seventeen days. A foreperiod of five days was followed by two bathing periods of four days each separated by an interim of two days without bathing. The lake water had a content of 19.2 per cent total solids of which 93 per cent were chlorides (as NaCl). The urine, collected in 24-hour periods, was analysed for total nitrogen, creatinine, uric acid and chlorides. Total nitrogen excretion in the two subjects shows an increase of 7 and 5 per cent during the bathing periods, over the amounts excreted in the preliminary and final periods, which is probably a true stimulation of nitrogen metabolism rather than a destruction of protein for fuel. Uric acid variations are small. Creatinine elimination shows a slight rise during the bathing periods, which, if significant, may be related to increased muscular tonus. Body temperature fell 1 to 2°F. as a result of bathing, falling most rapidly not during the bath but after it while drying. Chloride excretion was considerably increased during the bathing periods, in the two cases 21 per cent, and 27 per cent, over the amounts eliminated during the preliminary and final periods. These variations have no adequate parallel in those of any other catabolite determined and the possibility of absorption through the skin is suggested and discussed.

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